

**NUTRITIONAL STATUS AND IMMUNE FUNCTION
IN AN ELDERLY POPULATION**

CENTRE FOR NEWFOUNDLAND STUDIES

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NUTRITIONAL STATUS AND IMMUNE FUNCTION IN AN ELDERLY POPULATION

By

©Barbara Vera Roebothan, B.Sc., M.Sc.

A thesis submitted to the School of Graduate
Studies in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Faculty of Medicine
Memorial University of Newfoundland

August 1991

St. John's

Newfoundland



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ISBN 0-315-73320-9

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ABSTRACT

It has been suggested that nutrition and immunology are integrally related. It has also been suggested that many of the elderly have both a depressed immune response and a poor nutritional status. We proposed to improve the immune response of some nutritionally deficient seniors by improving their nutritional status.

205 healthy elderly volunteers were assessed for their nutritional status in regards to protein/calories, zinc, iron, folacin, and vitamin B₁₂. The assessment composed of anthropometric (height, weight, triceps skinfold, subscapular skinfold, and mid upper arm circumference), biochemical (serum albumin, serum prealbumin, and serum zinc), haematologic (serum ferritin, serum vitamin B₁₂, serum folacin, haemoglobin, and haematocrit), and clinical examinations. Dietary intake was also recorded.

66 (32.2%) of these individuals showed signs of malnutrition. 14 (6.8%) showed signs of multiple deficiency. Deficiencies of all nutrients monitored were found in the subject group. Protein/calorie malnutrition was the most prevalent at 13.2%. Folacin and vitamin B₁₂ deficiencies were the least prevalent, both at 2.4%. The prevalence of malnutrition did not differ with sex or living accommodation (institutionalized versus noninstitutionalized) but did increase significantly with age.

42 of the nutritionally deficient were administered the appropriate nutritional supplement for six consecutive months. Of these, 34 showed an improvement in nutritional status. A comparison of delayed cutaneous hypersensitivity, complement C3 levels, and percent of total lymphocytes represented by functional T cells, CD4+ cells,

and CD8+ cells was made in these individuals before and after the supplementation period. A significant rise in functional T cells was noted.

These findings support suggestions by work performed largely on animals and other aged groups of humans that nutrition can have a significant and positive effect on immune function.

INDEXING KEY WORDS : nutrition, elderly, cellular immunity

ACKNOWLEDGEMENTS

There are many individuals to whom I owe thanks for assistance in researching and composing this thesis. There are those who provided me with academic and technical assistance and there are those whose personal support enabled me to persevere.

First and foremost I would like to express my sincere gratitude to Dr. Ranjit Chandra. He is a great source of knowledge and this a student cannot do without but more importantly to me he is a man of patience and understanding. This is a side of him which many do not get the privilege to see but without this side I feel sure that this thesis would never have been completed.

I would also like to express thanks to the other members of my advisory committee, Dr. John Lewis and Dr. Bill Eaton, for their time, co-operation, and suggestions. Dr. James Friel graciously offered and painstakingly did proofread this thesis. Dr. Andrew Luong and Dr. David Bryant acted as my statistical consultants. I thank them all.

Technical assistance was given by many in the course of my work but special thanks should be given to Thekla Lundin, Karen Lever, and especially to Bing Au. Mr. Au not only introduced me to laboratory immunology but also provided much guidance in the actual compilation of the thesis itself.

Both Ross Laboratories and Health and Welfare Canada should be thanked for financial support without which this project could never have proceeded. Thanks should also be given to the Department of Biochemistry with its faculty and staff and the Faculty

of Medicine both of whom provided me with technical and academic assistance on many occasions.

Due to the complex nature of my personal life there are many friends and relatives who must also be thanked. First and foremost of these is my husband Glen who bore with me over these tumultuous years without ever receiving any real thanks until now. Also his parents should be thanked for their time and support which did not go unnoticed.

My family too gave me the necessary moral support which one in my situation needs to go on. Direct help was given by both my brother Bill and my sister Mona. Parents are always acknowledged at a time like this and I thank them both sincerely.

Finally I thank my four children Katie, Julia, Mark, and Paul for it was really their time which I spent in the preparation for and the compilation of this thesis. I hope that we will all benefit from it.

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LIST OF ABBREVIATIONS

AET	=	aminoethylisothiuronium bromide
AIDS	=	autoimmune deficiency syndrome
APC	=	antigen-presenting cell
BSS	=	balanced salt solution
°C	=	degrees Celcius
C	=	complement
CaCl ₂	=	calcium chloride
CD	=	cluster designation
cm	=	centimetre
ConA	=	concanavalin A
CRP	=	C reactive protein
DCH	=	delayed cutaneous hypersensitivity
DNCB	=	dinitrochlorobenzene
DTH	=	delayed type hypersensitivity
FCS	=	fetal calf serum
FITC	=	fluorescein isothiocyanate
g	=	gram
HANES	=	Health and Nutrition Examination Survey
HCl	=	hydrochloric acid
H ₂ O	=	water
Ig	=	immunoglobulin

IL	=	interleukin
KCl	=	potassium chloride
KH_2PO_4	=	potassium phosphate
l	=	litre
LPS	=	lipopolysaccharide
M	=	molar
mcg	=	microgram
ml	=	millilitre
mm	=	micrometer
MgCl_2	=	magnesium chloride
MgSO_4	=	magnesium sulfate
MHC	=	major histocompatibility complex
ml	=	millilitre
mm	=	millimetre
NaCl	=	sodium chloride
Na_2HPO_4	=	sodium phosphate
NaOH	=	sodium hydroxide
PBS	=	phosphate buffered saline
PCM	=	protein-calorie malnutrition
PEM	=	protein-energy malnutrition
PHA	=	phytohaemagglutinin
PMNL	=	polymorphonuclear leukocyte

pmol	=	picamole
PPD	=	purified protein derivative
RBC	=	red blood cell
RDI	=	recommended dietary intake
RNI	=	recommended nutrient intake
sIgA	=	secretory immunoglobulin A
sRBC	=	sheep red blood cells
w/v	=	weight per volume

CHAPTER 1

INTRODUCTION

1.1 AN AGING SOCIETY

Our society is getting older. Not only is the absolute number of older individuals in our society increasing but so is the percentage which they represent in our overall population.

Today approximately 15.5% of Canadians are above the age of 60 years (Statistics Canada, 1988). This represents a rise of 3% in just 12 years (12.6% in 1976) (Canadian Ministry of Supply and Services, 1980-1981). Similarly American statistics state that in the year 1900 only 4% of the U.S. population was above the age of 65 years. This rose to 12% in 1985 and is predicted to reach 25% by the year 2030 (U.S. Department of Health and Human Services, 1988).

It has been suggested that of this age group, it is the "elderly elderly" or those above the age of 80 years who will increase most in number (Leveille and Cloutier, 1986; Davies, 1990). Also the gap in life expectancy which we now experience in favour of the female will likely be maintained (U.S. Department of Health and Human Services, 1985; J.E.Brown, 1990).

This aging phenomenon is not limited to North America. Although certain areas of the developed world such as Canada and the United States are experiencing this trend at an escalated pace, it is being experienced worldwide (Wahlqvist and Kouris, 1990; Grundy, 1983; Exton-Smith, 1982; Morley, 1986).

1.2 AGING

What is aging? It is a process of change which is inevitable in living systems. Many see this process as one that begins soon after fertilization and which culminates in death.

There are many theories which attempt to explain the process of aging. Some see it primarily as an endocrine dysfunction, others as an immune dysfunction, some as a consequence of free radical damage, and still others as a program which each cell bears to set the maximum number of divisions which it can support (Wardlaw and Insel, 1990; Alford and Bogle, 1982; Masoro, 1990; Fudenberg, 1981; Finch, 1979; Kay and Makinodan, 1976).

It is generally acknowledged that cell aging is probably influenced by both automatic cellular changes (genetically programmed) and environmental influences. There are studies which support the genetic determination of longevity (Popp and Popp, 1981; Brown, 1990; Walford, 1974) but the effects of environmental factors are also well established (Brown, 1990; Kay and Makinodan, 1976; Halsall and Perkins, 1974). Most of the gains in life expectancy seen over the past few decades in North America are likely attributable to environmental factors.

A number of environmental factors have been identified but dietary restriction without malnutrition tends to be accepted as the one which has the most potential for control over the aging process (Wardlaw and Insel, 1990; Walford, Harris and Weindruch, 1987; Good and Lorenz, 1988; Good, West and Fernandes, 1980; Rabin, 1982; Yu et al. 1982; Good, 1981; Weindruch and Makinodan, 1981; Masoro, 1988).

Most research in this area has been carried out on rodents with weaning-initiated underfeeding but limited success has also been achieved with humans whose underfeeding began in adulthood (Rabin, 1982; Watson and Safranski, 1981; Weindruch and Makinodan, 1981) "... food restriction is the only manipulation that has been shown markedly and reproducibly to increase the maximum lifespan of a mammalian species..."(Masoro, 1990).¹

In man the changes which constitute the aging process are both numerous and varied. These include changes in both body composition and physiological function (Young, 1990; Munro, 1981; Roland, 1984; Alford and Bogle, 1982). Such composition changes generally represent a loss of active tissue mass and as mass declines so does the function of organs and tissues (Munro, 1981).

Aging brings a reduced ability to compensate for physiological stress. This is why aging begins very early but its consequences may not be seen for some time. The detrimental pressures of aging may be experienced by cells at day one but the organs in which these cells function retain enough reserve capacity that they can function for a long time without showing any ill effects or signs of disease. Only with further aging and the exhaustion of the reserve capacity is the actual decline in organ function apparent (Wardlaw and Insel, 1990).

1.3 HEALTH STATUS OF THE ELDERLY

With the older segment of our population growing in size their health status will

¹ Masoro EJ. Physiology of Ageing: Nutritional Aspects. Age and Ageing 1990: 19(4),6.

closer represent our society's health status as a whole and this is an unfortunate situation since the elderly experience a higher prevalence of disease than any other age group (Chandra, 1990; Exton-Smith, 1982).

The decline in bodily functions which tends to accompany the aging process predisposes the aging body to such disease states as cancer, heart disease, autoimmune disease, and cerebrovascular disease. It has been well established that the prevalence of such degenerative diseases is highest in the elderly (Exton-Smith, 1982; Samiy, 1983; Fudenberg, 1981).

The elderly also experience a high rate of infectious disease (Linn, 1987; Berk and Smith, 1983; Schneider, 1983; Vitale and Santos, 1982; Hill and Stamm, 1982; Galpin, 1981; Yoshikawa, 1983; Ebright and Rytel, 1980; Parratt, 1980). For example, in the United States pneumonia and influenza constitute the fourth major cause of death in those over five years of age (Ebright and Rytel, 1980) and of those patients with pneumonia who require hospitalization, approximately 50% are at least 60 years of age (Hill and Stamm, 1982). Other infections of specific importance to the elderly include urinary tract infections, tuberculosis, pyoderma, and herpes zoster (Yoshikawa, 1983). Although anecdotal knowledge suggests that a distinctive pattern of infections is found in the elderly, not enough sound research has been done in this area to allow us to define such a pattern as yet (Schneider, 1983).

The prevalence of infections in the elderly is high. It is also important to note that the aged human is not only more susceptible to certain infections but that he/she also experiences a higher morbidity and mortality associated with these (Galpin, 1981).

Many attribute the high susceptibility of disease in the elderly to a depressed immune function. More and more data is being found to support this (Schneider, 1983). There are likely other contributing factors such as nutrition. Indeed many of the diseases common to the elderly are nutrition related diseases (Wardlaw and Insel, 1990) and it has been claimed by some that a number of the infections experienced by the elderly have been precipitated by malnutrition (and vice versa) (Vitale and Santos, 1982).

1.3.1 Distribution of Health Problems

The more knowledge which we accumulate on the functioning of the human body the more obvious it becomes that individuals can be quite different. This applies to the elderly as well as to other age groups. The growing number of elderly is continuously adding to the heterogeneity of this group.

The aged community is a unique group in many ways but is yet extremely heterogeneous (Schneider et al. 1986; Rivlin, 1982; Masoro, 1990). The aging process affects different people at different rates, to different extents, and in different ways. For example, the decline in immune function which is seen with age is an average response. The average immune response of the elderly is lower than the corresponding average response measured in younger adults yet the range is much broader. Actually a proportion of elderly maintain quite a vigorous immune response (Chandra, 1990). This probably holds true for many bodily functions and thus disease states. Just because one reaches a certain chronological age does not mean that that individual's body can be expected to function at a predictable level of competence. Indeed the older one becomes

probably the less predictable one becomes. Consequently, disease prevalence may be higher in the elderly but this by no means should suggest that the health status of all elderly individuals is compromised.

Heterogeneity of the elderly is apparent in many other ways. The issue of living accommodations provides a categorization of senior adults which is much less obvious in other age groups. There are probably more individuals institutionalized in their senior as compared to their younger years. It has been suggested that the institutionalized elderly differ from the noninstitutionalized elderly in health status and specifically in risk of malnutrition (Pinchcofsky-Devin and Kaminski, 1987; Rosenberg et al. 1982).

1.3.2 Practical Implications

The growth in numbers of the elderly will demand a change in our health care system. As our population ages so our health care system will have to change and concentrate more on the specific health problems suffered by the elderly. Not only will the type of care demanded by our society change but so will the amount of health care since the elderly constitute that segment of our population with most health problems. Unless changes are made to improve the health status of the elderly, health care costs can be expected to rise continuously with time.

1.4 IMMUNOLOGY

A competent immune system is an absolute necessity for the human body to keep abreast of the enormous array of microorganisms to which it is continuously exposed

(Parratt, 1980). Yet, research suggests that the functioning of the average immune system steadily decreases with increasing age (Kay and Makinodan, 1976; Callard, 1981; Katz, 1982; Nordin and Makinodan, 1974; Leech, 1980). Some claim that progressive dysfunctions of the immune response may be a primary cause of aging (Homsy, Morrow and Levy, 1986; Fudenberg, 1981; Kay and Makinodan, 1976; Finch, 1979) and consequently the immune system may be the most attractive target available for aging studies (Mullen, 1981; Weksler, 1980; Kay and Makinodan, 1976; Makinodan and Adler, 1975).

What is a normal and adequate immune response and how is it compromised in the elderly?

1.4.1 Host Defense Mechanisms

The immune system equips the body with a defense against potentially harmful invaders or antigens but the first line of defense offered by the body is made up of various nonspecific physical barriers such as skin and mucous membranes (Neumann, 1977; Heggers, 1979; Chandra, 1981c). When intact, the skin provides the body with a good barrier against the outside world. The skin also secretes many fluids, some of which contain active antimicrobial agents such as lysozyme, an enzyme which hydrolyzes the acetyl-amino polysaccharide constituent of bacterial cell membranes (Heggers, 1979; Chandra et al. 1977). Similarly the mucous membranes if not damaged prevent penetration of potentially pathogenic organisms into deeper recesses of the body such as the gastrointestinal and respiratory tracts. The secretions of the mucous membranes also

contain antimicrobial substances.

When something gets past this primary line of defense then the immune system must respond. Very minimal criteria need to be met before the body will identify something as an antigen and mount an immunologic response. These include such antigenic characteristics as being at least macromolecular in size and bearing "proof" of nonself (Roitt, 1980).

The immune response is subdivided as innate and adaptive with innate referring to general responses geared towards no specific antigen while an adaptive response is triggered only in the presence of the specific antigen to which it is sensitive (Roitt, Brostoff and Male, 1989). Each of these subdivisions has both a cellular and a humoral component.

1.4.1.1 The Innate Immune Response

All antigens in the body are exposed to various soluble factors which are made by the body and circulated in an attempt to damage any susceptible antigens in the body with which they come in contact. These nonspecific soluble factors include lysozyme, complement, and acute phase proteins such as C-reactive protein (named after its ability to bind the C protein of pneumococci) and interferon.

Acute phase proteins are a heterogeneous group of proteins, mostly glycoproteins, whose serum concentrations rise quickly with infection and remain elevated only throughout the duration of that infection (Roitt, Brostoff and Male, 1989; McFarlane, 1977; Dionigi, 1982).

Complement refers to a group of approximately 20 proteins, many of which are acute phase proteins. Complement is capable of binding to many different antigens and facilitating the engulfment of such antigens by nonspecific phagocytes. Opsonization is the term which refers to the protein coating of an antigen to promote its phagocytosis. The complement system is responsible for many activities other than opsonization but to function it must be activated. This activation can be achieved either by the classical or alternative routes. Once activated the complement system has the capability of performing many functions which largely help to control inflammation (Roitt, Brostoff and Male, 1989; Keusch, 1981; Keusch, 1982).

The cellular aspect of the innate immune response is composed of a variety of phagocytic cells, each one of which is capable of engulfing various antigens. All of these phagocytic cells originate in the bone marrow of the adult from a common stem cell. This stem cell also gives rise to lymphoid cells which participate in immune responses mounted after specific antigens -T lymphocytes and B lymphocytes (Lipschitz, 1987; Roitt, 1980; Roitt, Brostoff and Male, 1989) (appendix A).

The vast majority of nonspecific phagocytes are collectively referred to as the polymorphonuclear (PMN) cells or granulocytes. Histologically these can be subdivided into numerous categories but all of these function to phagocytize foreign particles and/or cells to which they are exposed.

Another group of immunological cells with a similar function are the mononuclear monocytes and macrophages. They differ from one another largely in size and location in the body (Volkman, 1976). They differ from the other nonspecific phagocytes not only

because of their nucleus but also because of number. There are much fewer of these in comparison to the PMN cells, and they may be somewhat less general in regards to the antigens that they attack (Roitt, 1980; Stinnett, 1983).

1.4.1.2 The Adaptive Immune Response

If an antigen does get access to the body and the innate line of defense has not been entirely successful at dealing with the situation the body is equipped with yet another line of defense, again with both a cellular and a humoral component. In this case different cells are triggered by different antigens. The cellular aspect of the adaptive response tends to deal primarily with intracellular antigens such as viruses, mycobacteria, protozoa, and fungi while the humoral or noncellular aspect concentrates on extracellular infections such as those by the pyogenic bacteria (Edelman, 1977).

The T and B lymphocytes respond to specific antigens. These cells originate from the same stem cells as the PMN cells, monocytes, and macrophages (appendix A) but they develop quite differently from each other (Keusch, 1981; Roitt, 1980; Roitt, Brostoff and Male, 1989).

A common precursor of the lymphocyte is produced in the bone marrow in man. Most of these precursors travel to the thymus in the upper mediastinum where they undergo preliminary development. These lymphocytes bear the designation T after the thymus since this is the lymphoid organ where they underwent early development - their primary lymphoid organ. The functioning of the thymus is believed to be under the control of hormones and possibly other factors (Roitt, Brostoff and Male, 1989; Horrobin

et al. 1979; Dardenne et al. 1982).

Some of the precursor cells actually remain in the bone and undergo early development there. They are referred to as the B cells and it is these which will ultimately give rise to the specific humoral response.

The T and B lymphocytes are released into circulation but do not further differentiate into fully developed functional cells unless they are exposed to the specific antigen to which they are set to respond. If that antigen is in the body it must be initially processed and properly presented to the appropriate lymphocyte in order for the lymphocyte to become activated. Processing is done by a heterogeneous group of cells which includes macrophages and maybe even some B cells (Roitt, Brostoff and Male, 1989). These cells probably internalize the antigen and process it in such a way as to expose a portion of it, probably a small peptide, to nearby responsive cells. Consequently these processors are referred to as antigen-presenting cells (APC). APC's also produce molecules involved in the activation of lymphocytes. The best studied of these is probably interleukin-1 (IL-1). It is likely that the T and B peripheral lymphocytes bear some sort of surface adhesion molecule so that they can home in on "receptor structures" in locations where the antigen-presenting cells tend to be found (Roitt, Brostoff and Male, 1989).

The secondary or peripheral lymphoid organs include the lymph nodes, spleen, tonsils, Peyer's patches, and nonencapsulated aggregates of lymphoid tissue found in such sites of the body as the gastrointestinal, urogenital, and respiratory tracts. These create an environment where lymphocytes can interact with each other and with antigens. Each

lymphocyte can recognize only one antigen. That antigen binds to the small number of cells capable of interacting with it. Once activated by the appropriate antigen the lymphocyte proliferates into a clone of cells big enough to mount an effective response and further develops in the secondary lymphoid organ into a mature effector cell or a memory cell. A small number of the latter are retained by the body so that a response to an exposure to the same antigen in the future can be both quicker and more pronounced.

Both the activation of T and B lymphocytes and their functioning as fully differentiated mature cells requires a lot of interaction between themselves and other accessory cells (Callard, 1981; Heggors, 1979; Good, 1977; Santos, Arredondo and Vitale, 1983; Walford, 1974). Indeed the activation process itself includes an increased expression of a number of surface molecules allowing more interaction with other cells (Roitt, Brostoff and Male, 1989).

Processed antigens can activate specific T or B cells but they can also be activated in vitro by a mitogen. Mitogens are polyclonal activators but different cells are more sensitive to some mitogens than others. Phytohaemagglutinin (PHA) and concanavalin A (Con A) preferentially stimulate human and mouse T cells while lipopolysaccharide (LPS) stimulates mouse B cells (Leech, 1980; Roitt, Brostoff and Male, 1989).

1.4.1.2.1 Functioning of the Effector Cells

The mature T cell is ultimately responsible for the specific cellular immune response while compounds produced by mature B cells are responsible for the specific

humoral immune response. The two cell types are highly interactive. B cells are especially dependent upon the presence of certain T cells to function although a few antigens provoke an effective response from B cells which is T-cell independent.

1.4.1.2.1.1 T Cells

Mature T cells are subdivided according to the presence or absence of certain cell surface markers. T cells bear either antigen receptor TCR-1 or TCR-2 and can be subdivided on this basis. Of those cells expressing TCR-2, some express the surface molecule CD4 (cluster designation 4) and those which do not usually express the surface molecule CD8 (appendix B). Many other CD molecules have been characterized but are of no value to use in subdividing T cells by function (Roitt, Brostoff and Male, 1989).

TCR-2 CD4+ have been referred to as T_4 , T-helper, or T_h cells because of their assistance to other cells of the immune system such as the B cell. TCR-2 CD8+ have been labelled as T_8 , T_c , or cytotoxic/suppressor cells and they exert a much more negative effect. They have the ability to suppress certain immune responses (Linn, 1987; Good, 1977; Roitt, Brostoff and Male, 1989).

The Major Histocompatibility Complex (MHC) is a group of genes which code for specific proteins of importance to immune function (Popp and Popp, 1981; Roitt, Brostoff and Male, 1989). In man the MHC is designated as the HLA gene cluster and is found on chromosome 6. The molecules encoded by this region can be divided into class I, class II, and class III. The molecules of the first two classes are of particular

importance for immune recognition. Most helper T cells not only are CD4+ but also recognize antigens in association with class II MHC molecules. Most cytotoxic/suppressor T cells are both CD8+ and able to recognize antigens in association with class I MHC molecules (Roitt, Brostoff and Male, 1989). This delineation is good but not perfect.

TCR-1 cells can be separated into two categories according to whether the cell bears the surface marker CD8 or not (appendix B). All TCR-1 cells appear to perform a somewhat nonspecific killer cell function (Roitt, Brostoff and Male, 1989).

Some activated T lymphocytes possess their own effector function (for example, cytotoxic T cells) but many synthesize and release factors which allow them to communicate with other cells without having to physically contact them. These mediators, usually protein molecules, are lymphokines and can exert both positive and negative effects on the functioning of various immune cells including macrophages and B lymphocytes.

Occasionally an adaptive immune response occurs in an exaggerated form and may even cause tissue damage. This hypersensitivity reaction is manifested on the second contact with the antigen. Hypersensitivity reactions have been generally classified into four types one of which is the delayed type hypersensitivity (DTH). Delayed type hypersensitivity is characterized by the response manifesting itself some time after the antigen challenge. The time lapse involved is usually 24 - 72 hours but can be considerably longer.

T cells appear to be responsible for this phenomenon (Gross and Newberne, 1980;

Roitt, 1980; Roitt, Brostoff and Male, 1989; Tyan, 1981; Cunningham-Rundles, 1982).

It is the T cells which have been sensitized to the antigen by a previous exposure and it is the T cell and the other cell types which it recruits which support the characteristic exaggerated response.

1.4.1.2.1.2 B Cells

Ultimately most "invaders" of the human body are disposed of by phagocytes and/or by activated complement components. The situation could exist where a potentially dangerous antigen gained access to the body but could not be recognized by phagocytes nor activate complement. Antibodies have evolved to deal with such a situation. Antibodies form the basis of the adaptive humoral response.

Much like T cell activation and proliferation, B cells multiply and differentiate into mature plasma cells when exposed to the appropriate antigen. Most B cells recognize processed antigens in combination with MHC class II molecules and usually in cooperation with T cells (Roitt, Brostoff and Male, 1989). Most cells resulting from the proliferation are effector cells but some are retained as memory cells.

Plasma cells synthesize and secrete antibodies. Each plasma cell can produce thousands of molecules of antibody but every molecule from that cell will be identical. Antibody molecules from any plasma cell derived from the same clone will also be identical. They are all specific for the antigen which triggered their production.

Antibodies are immunoglobulins (Ig). As such they can be classified into IgA, IgD, IgE, IgG, and IgM. The roles of each can be distinguished as can the structures but

the structures of all are based upon a "hinged" molecule of four distinct chains held together by disulfide bonds (appendix C) (Stinnett, 1983; Roitt, 1980). Two of the four chains are heavy chains (due to their high molecular weight) and two are light chains. Each chain has both constant and variable regions. Constant regions share a relatively high degree of homology of amino acid sequence with other immunoglobulin chains. Variable regions differ considerably from one another and thus allow for the enormous repertoire of antibodies which any individual has the potential to house.

Antibodies function to bind antigens with phagocytes and/or activated complement components, both of which have the capability of destroying the antigen. Sites on the immunoglobulin molecule have been identified as being responsible with these distinct roles of the antibody. Each molecule bears two antigen binding sites each one of which is composed of a variable region of a light chain in close proximity to a variable region of a heavy chain (Stinnett, 1983; Roitt, 1980).

Antigens have specific surface markers to which antibodies bind. That part of the antigen to which an antibody binds is the antigenic determinant or the epitope. One antigen can bear several epitopes. These epitopes may or may not be identical (Roitt, Brostoff and Male, 1989).

The physical bridging of antigens with phagocytes is how antibodies function to rid the body of the potential harm which the antigen could otherwise cause. To ensure that the phagocytosis will be effective the antibody also has the capability of interacting with complement. Activated complement attracts phagocytes to the point of infection but also increases blood flow to the site and thus a supply of accessory immune cells which

are in circulation, and increases the permeability of capillaries to these plasma molecules. In addition certain components of the complement cascade can themselves directly damage cell membranes leading to cell lysis (Roitt, Brostoff and Male, 1989).

1.4.1.3 Summary

The immune system is a highly integrated and complex system which does not easily lend itself to simple definitions and explanations. There are so many components to this system that no attempt at explaining it can ever be successful at including them all. The intricate interrelationships between its components both cellular and humoral must always be kept in mind in order for one to really appreciate the true functioning of this system. The immensity of its value to human existence may never be appreciated.

1.4.2 Immune Senescence with Aging

There have been some difficulties in establishing how the normal immune system truly behaves in the elderly (Fudenberg 1981) but it has been accepted that on average, the immune response of the aged is less aggressive than that of younger persons (Thompson, Robbins and Cooper, 1987; Katz, 1982; Mackay, 1972; Roberts-Thompson et al. 1974). Although this has been shown to be generally true, a minority of seniors do retain a more youthful pattern of immune function (Chandra, 1990). In most cases however both the cellular and humoral immune responses are likely compromised to some extent (Callard, 1981) but the biggest effect is on the cellular immune response (Chandra, 1990; Lipschitz, 1987; Makinodan and Adler, 1975; Popp and Popp, 1981;

Thompson, Robbins, and Cooper, 1987; Makinodan, Lubinski and Fong, 1987).

1.4.2.1 Effects on the Adaptive Cellular Response

Atrophy of the thymus gland is probably the key to aging of the immune system. It is certainly of major importance to aging of the cellular aspect of the immune response (Lipschitz, 1987; Kay and Makinodan, 1976; Gershwin, Beach and Hurley, 1983).

1.4.2.1.1 The Lymphoid Organs

At sexual maturation the thymus begins to atrophy (Katz, 1982). Its involution and accompanying decrease in size begin at about 15 years and progress rapidly during adulthood so that by approximately 50 years its mass is significantly less and the remaining tissue is largely vestigial (Mackay, 1972; Katz, 1982; Popp and Popp, 1981; Kay and Makinodan, 1976; Lipschitz, 1987).

Secondary lymphoid organs such as the lymph nodes and spleen appear to maintain their size with progressing age (Popp and Popp, 1981; Makinodan, 1977; Kay and Baker, 1979) or lose very little mass (Katz, 1982). Structural changes though may occur (Katz, 1982).

Why does the thymus atrophy with progressing age? The cause may be intrinsic, possibly encoded in the DNA (Kay and Makinodan, 1976). Alternately it could be extrinsic and due to a breakdown of the hypothalamus-pineal-pituitary neuroendocrine axis as it pertains to regulatory control over the thymus (Fudenberg, 1981; Kay and Makinodan, 1976; Finch 1979).

1.4.2.1.2 Thymic Hormone

The thymus is an endocrine organ. The thymic hormone(s) or factor(s) which it secretes is necessary for the proper maturation and differentiation of the T cells. The production of thymic hormone or thymulin is diminished and its concentration in the serum drops with thymic atrophy (Goldstein et al. 1979; Tyan, 1981; Thompson, Robbins and Cooper, 1987; Fabris et al. 1984). By the age of 65 years the activity of thymic hormone is almost undetectable (Chandra, 1990). This is at least partly responsible for the elevated number of immature T cells seen in the elderly (Thompson, Robbins and Cooper, 1987; Lipschitz, 1987).

Other factors such as interleukin-2 may also aid in T cell maturation. The serum concentration of interleukin 2 (IL-2) is depressed in the elderly and may therefore also contribute to the high levels of immature T cells seen (Chandra, 1990; Thompson, Robbins and Cooper, 1987; Weksler, 1983).

1.4.2.1.3 T Cells

A change in both the number of circulating T cells and the structure/function of these cells has been suggested in the elderly.

1.4.2.1.3.1 Structural and Functional Changes

Both surface and internal structural changes have been noted in T cells from the elderly. The type and number of receptors on these cells may vary with age (Chandra,

1990; Makinodan and Lubinski, 1987). Although the amount of "theta" antigen expressed on the surface of T cells diminishes with age (Kay and Makinodan, 1976) a new antigen is expressed - the "senescent cell antigen" (Chandra, 1990). Internally "...there is swelling of mitochondria and presence of myelin-like structures and reduced number of cristae in cells from old individuals."(Chandra, 1990)².

Metabolic changes may also occur within the cells. Levels of cAMP and cGMP appear to be altered in both resting and mitogen-stimulated T cells (Chandra, 1990). This has also been seen in mice (Kay and Makinodan, 1976).

T cells from the elderly tend to have a lowered response to mitogens (Popp and Popp, 1981; Makinodan and Adler, 1975; Roberts-Thompson et al. 1974). This has been fairly well established with the mitogen phytohaemmagglutinin (PHA) in both animals and man (Lipschitz, 1987; Thompson, Robbins and Cooper, 1987; Katz, 1982; Callard, 1981; Kay and Makinodan, 1976; Hallgren et al. 1973; Pisciotto et al. 1967). Work with mice suggests that the T cell surface receptors for PHA are intact and functional but a problem lies in the control of the process possibly by thymic hormone (Lipschitz, 1987; Kay and Makinodan, 1976). The low levels of thymic hormone are at least partly to blame since the response of these cells to PHA is restored when the old T cells are exposed to thymic hormone (Lipschitz, 1987; Wittingham and McKay, 1973).

1.4.2.1.3.2 Changes in Number

Immature T cells may be found in elevated numbers (Lipschitz, 1987; Thompson,

² Chandra RK. The Relation between Immunology, Nutrition, and Disease in Elderly People. Age and Ageing 1990; 19:s28.

Robbins and Cooper 1987; Weksler, 1983). The number of mature T cells circulating in the elderly is probably normal (Lipschitz, 1987; Thompson, Robbins and Cooper, 1987; Popp and Popp, 1981; Hallgren et al. 1978; Makinodan and Adler, 1975; Stutman, 1974; Weksler and Hutteroth, 1974) or slightly depressed (Chandra, 1990; Smith, Evans and Steel, 1974) but some claim that this number is significantly less than what is seen in younger adults (Katz, 1982; Tyan, 1981; Kay, 1980; Czlonkowska and Korlak, 1979; Reddy and Goh, 1979; Girard et al. 1977; Carosella, Mochanko and Braun, 1974; Mackay, 1972). Some even suggest that the number of functional T cells may increase in the elderly-elderly (Hallgren et al. 1978; Hallgren et al. 1974).

Claims have been made that the elderly have a lower T helper activity (Lipschitz, 1987; Thompson, Robbins and Cooper, 1987; Makinodan and Adler, 1975; Nordin and Makinodan, 1974) and an elevated T suppressor activity (Lipschitz, 1987; Callard, 1981) as compared to younger adults. This may be due to a redistribution of T cell subsets with aging. How the cells may be redistributed is as yet not clearly established. It has been suggested that the number of CD4+ cells (T helper cells) in the aged is maintained or slightly reduced (Chandra, 1990) although Weksler does not agree (Weksler, 1983). Chandra has shown that the percentage of total lymphocytes represented by CD4+ cells drops somewhat with aging and that an even larger drop is seen in the percentage of total lymphocytes represented by CD8+ cells (T suppressor cells) (Chandra et al. 1982). Weksler agrees with the drop in CD8+ cells (Weksler, 1983) although not all do (Thompson, Robbins and Cooper, 1987). Makinodan's opinion after reviewing the relevant literature, is that both subpopulations decrease to a similar extent

with time and therefore their proportion does not appreciably change with age (Makinodan and Lubinski, 1987). As yet it is difficult to sort out this conflict in the literature (Cassavant and Stites, 1981).

1.4.2.1.3.3 Physiological Changes

Physiological changes in the aged which may reflect reduced T cell number and/or function include a lowered alloreactivity of graft versus host (Thompson, Robbins and Cooper 1987; Callard, 1981) and suppressed delayed hypersensitivity reactions (Popp and Popp, 1981; Roberts-Thompson et al. 1974; Waldorf, Wilken and Decker, 1968; Gross, 1965). A reduced delayed cutaneous hypersensitivity (DCH) response to purified protein derivative (PPD) (Czlonkowska and Korlack, 1979), Candida, mumps (Halgren, Jackola and O'Leary, 1983; Lichtenstein et al. 1982) and other recall antigens (Lipschitz, 1987) as well as to dinitrochlorobenzene (DNCB) (Chandra, 1990; Katz, 1982) is seen. The dermal response is decreased in both size and number.

DCH is a complicated process and involves the functioning of more than T cells. It has been visualized by some as being composed of at least three separate limbs - the sensitization or afferent limb; the recognition, recall, or efferent limb; and the inflammatory limb (Edelman, 1977; Waldorf, Wilkens and Decker, 1968). The fault seen in the DCH response of the elderly may be due primarily to the afferent limb (Edelman, 1977; Waldorf, Wilkens and Decker, 1968). There is some suggestion that delayed hypersensitivity which developed at an earlier age is essentially maintained but that an elderly individual might be more likely to exhibit anergy (no response) to a new allergen.

This is how Waldorf, Wilkens, and Decker explain their finding that those above 70 years of age responded less well to DNCB as compared to those less than 70 years (Waldorf, Wilkins and Decker, 1968). DNCB is an antigen that one is unlikely to have contacted naturally in the past. This would also tend to support the theory that the afferent limb of this response loses its effectiveness with aging.

Grossman claims that it is the acute illness so often experienced by the elderly which is responsible for their depressed DCH and not their advanced age (Grossman et al. 1975). They claim that 88% of the elderly, if not sick and if tested with a battery of antigens, would react with a positive response to at least one antigen.

1.4.2.2 Effects on the Adaptive Humoral Response

Most of the decline in immune function seen with aging can be accounted for by the involution of the thymus, the resultant drop in thymic hormone(s), and the effects that this has on the T cell but changes in the humoral response have also been noted.

1.4.2.2.1 B Cells

Although there is no evidence to suggest a change in B cell number or structure there is some support for a reduced functioning of these cells in certain respects in the older individual.

1.4.2.2.1.1 Changes in Number

B cell enumerations have been performed on subjects of various ages. These

suggest that B cell number is maintained regardless of age (Weksler, 1983; Katz, 1982; Popp and Popp, 1981; Kay and Makinodan, 1976; Makinodan and Adler, 1975; Stutman, 1974). When expressed as percentage of total lymphocytes the B cells still appear to be maintained (Mullen, 1981).

1.4.2.2.1.2 Functional Changes

There is some support for a decreased rate of proliferation of B cells in response to mitogens (Popp and Popp, 1981; Tyan, 1981; Roberts-Thompson et al. 1974). This loss of function could be minimal (Chandra, 1990).

Lipschitz suggests that the rate of antibody production by activated B cells is also depressed with age (Lipschitz, 1987). Several researchers have noted changes in antibody levels in the elderly.

1.4.2.2.1.2.1 Antibodies

There is some dispute as to whether total immunoglobulin levels are maintained in the elderly (Katz, 1982; Czlonkowska and Korlack, 1979) or lowered (Gershwin, Beach and Hurley, 1983; Goldstein et al. 1979). It has been suggested that only antibodies which require T cell assistance for their production are affected and therefore any change in antibody titre seen in the elderly is simply a reflection of the depressed cellular immune response (Chandra, 1990).

Weksler suggests that although the overall level of immunoglobulins in circulation is maintained that there is a redistribution of classes (Weksler, 1983). Levels of IgM may

drop (Weksler 1983) but levels of IgA probably rise (Chandra, 1990; Weksler, 1983; Katz, 1982). Accounts of both elevated (Weksler, 1983; Katz, 1982) and depressed levels (Chandra, 1990; Mackay, 1974) of IgG have been documented. Varying levels of this immunoglobulin in the elderly could reflect their susceptibility to infectious illness.

A number of researchers have reported increases in levels of autoantibodies circulating in the elderly (Lipschitz, 1987; Weksler, 1983; Gershwin, Beach and Hurley, 1983; Cunningham-Rundles, 1982; Rabin, 1982; Mullen, 1981; Popp and Popp, 1981; Leech, 1980; Goldstein et al. 1979; Hallgren et al. 1973; Rowley, Buchanan and Mackay, 1968). The true explanation for this phenomenon of an individual increasing the synthesis of antibodies against its own body parts with progressing age is unknown (Kay and Makinodan, 1976) but numerous theories have been presented. Some of these encompass the idea that a young individual has efficient control mechanisms which enable her/him to clearly differentiate self from nonself yet these mechanisms lose their efficiency with progressing age (Leech, 1980; Teague and Friou 1969). Greenberg and Yunis suspect that man's control over immune function and thus autoimmunity is governed by genes which may be linked with HLA (Greenberg and Yunis, 1978).

The rise in autoantibodies is accompanied by a drop in natural antibodies, antibodies against exogenous antigens (Weksler, 1983; Popp and Popp, 1981; Rowley, Buchanan and MacKay, 1968). In the elderly there is a lowered response to exogenous antigens (Weksler, 1983; Rabin, 1982). In both man and mouse, more antigen is required by the elderly to elicit a maximum response (Popp and Popp, 1981; Kay and Makinodan, 1976; MacKay, 1972; Price and Makinodan, 1972) and there is some suggestion that the

response could be slower (Chandra, 1990).

There are other findings which suggest that antibody function might be affected by aging. Weksler feels that the antibody response is of a shorter duration in seniors (Weksler, 1983). Katz notes that elevated levels of immune complexes (antigen-antibody) may circulate in the elderly (Katz, 1982) yet Chandra comments on the decreased affinity of antigen for antibody. He also notes that in some old animals the primary antibody response is low but that the antibody titre achieved after a booster is normal (Chandra, 1990).

1.4.2.3 Effects on Accessory Factors/Cells

Generally this area of research has received little attention. Those findings which have been made suggest a change here similar to that of the humoral response - cell numbers are essentially maintained but there may be minimal losses in function.

Phagocytosis is probably normal regardless of age (Chandra, 1990; Palmblad and Haak, 1978) but there is some scant evidence to suggest a possibility of minor malfunctions. The metabolic burst which accompanies intracellular killing of the phagocytosed particle/cell may be depressed in some phagocytes from older individuals (Chandra, 1990). It has been suggested that chemotactic activity may drop (Chandra, 1990; Corberand et al. 1981). Intracellular killing of *Candida* once ingested may (Corberand et al. 1981) or may not (Chandra, 1990; Palmblad and Haak, 1978) be affected.

Monocyte number and function seem to be maintained (Thompson, Robbins and

Cooper, 1987; Leech, 1980). Macrophage function is likely normal (Leech, 1980; MacKay, 1972) although there is some evidence in mice that their ability to present antigen may not be optimal (Popp and Popp, 1981; Makinodan and Adlar, 1975). Macrophage number is probably maintained (Leech, 1980) although Popp and Popp noted that an increased number of macrophages can be seen in some elderly (Popp and Popp, 1981).

Those complement components which have been measured in the elderly seem to be maintained (Vitale and Santos, 1985; Palmlad and Haak, 1978; Phair et al. 1978).

Natural killer cell levels may increase with aging (Thompson, Robbins and Cooper, 1987).

1.4.2.4 Effects on Stem Cells

Although there is little change in the number of most types of immune cells with the aging process there may be significant changes in T cell number. The function of T cells changes with aging and there is increasing evidence to suggest that the functioning of many other immune cells could also change. Since all of these cells originate from the same stem cell it could be deduced that changes in the proliferation and development of these stem cells could be in part responsible for these phenomena.

Changes in the stem cell population have been noted by some researchers in the aged, although much of this work has been limited to animals. The stem cell pool appears to be maintained but some functional properties of these cells may change with aging (Lipschitz, 1987; Makinodan and Adlar, 1975; Mackay, 1972; Mauch et al. 1982).

Some evidence is available to suggest that stem cells from older individuals undergo a decreased rate of proliferation and differentiation (Kay and Makinodan, 1976; Makinodan and Adlar, 1975). Whether this contributes to the possible drop in number of more mature immune cells such as the T lymphocyte has not been established.

It has been suggested that the total number of lymphocytes in circulation decreases progressively during and after middle age (Katz, 1982) although this is contested to some degree (Weksler, 1983). The percentage of these lymphocytes which are represented by T cells and by B cells may both be maintained (Greenberg and Yunis, 1978) but this also does not go without dispute (Leech, 1980).

Lipschitz agrees with a progressive drop in the total number of circulating lymphocytes with increasing age but claims that this is of limited practical significance since although the pluripotential hematopoietic stem cells have a limited lifespan and proliferative capacity they have been shown to maintain hematopoiesis beyond the maximum life expectancy of any of the animals yet studied (Lipschitz, 1987; Harrison, 1975).

1.5 NUTRITION

It is well accepted that nutrition is very important in the maintenance of good health. One's nutritional status changes with such influencing factors as age and disease state. It has been suggested that both old age and immune dysfunction may be associated with a depressed nutritional status. To observe any possible changes in nutritional status which accompany such factors it is first necessary to understand what good nutritional

health is for the young, healthy individual.

1.5.1 Nutritional Status of the Healthy Young Adult

A healthy young adult with no known physiological abnormalities and leading an acceptable lifestyle should be able to maintain his/her nutritional status by consuming appropriate amounts of the nutrients which have now been accepted as being essential for life (Health and Welfare Canada, 1990; Wardlaw and Insel, 1990). Many countries have specified what are appropriate amounts of each of the essential nutrients for their population to consume. In Canada these consumption guidelines are the Recommended Nutrient Intakes (RNI) (Health and Welfare Canada, 1990).

The nutrients which have been established as essential for life are protein, carbohydrate, lipid, 13 vitamins, and at least 22 minerals (Guthrie, 1989; Wardlaw and Insel, 1990; Hunt and Groff, 1990; Whitney and Hamilton, 1987). At least part of the function for most of these nutrients has now been established.

1.5.1.1 The Energy-Yielding Nutrients

Three of the essential nutrients provide the body with energy. These are carbohydrate, protein, and lipid.

The most densely caloric of the three is lipid or fat. Dietary fat not only provides the body with much needed energy but also with a supply of the essential fatty acids, linoleic and alpha linolenic acids. These are constituents of some dietary fats and are absolutely necessary by the body in small amounts to function properly. Without

adequate supplies not only will growth be affected but also skin abnormalities and gastrointestinal upset may develop (Wardlaw and Insel, 1990). The incidence of infectious illness may also rise (Whitney and Hamilton, 1987).

Protein too can and is used by the body as an energy supply. The body's top priority is energy but once it has an adequate supply then any additional protein consumed can be digested into its constituent amino acids and these are used to build the body's own proteins. The body synthesizes and utilizes a vast array of proteins, some structural and some functional (Whitney and Hamilton, 1987; Wardlaw and Insel, 1990; Guthrie, 1989).

Carbohydrate is the body's major supplier of energy. It is the least expensive dietary source of energy and today approximately 50% of the calories in the diet of the average Canadian are contributed by carbohydrate (Health and Welfare Canada, 1990). Carbohydrate can be replaced as a source of energy by fat or protein but undesirable symptoms can result when there is no carbohydrate in the diet (Guthrie, 1989). Due to the results of many population studies it has recently been suggested that some of our fat intake should be substituted by carbohydrate, especially complex carbohydrates (Health and Welfare Canada, 1990). High intakes of total and saturated fats have been associated with a higher risk of heart disease while high intakes of complex carbohydrates, including fibre, have been associated with a decreased risk of diseases such as colonic cancer and diverticular disease (Health and Welfare Canada, 1990; Wardlaw and Insel, 1990; Guthrie, 1989; Whitney and Hamilton, 1987). These associations have largely been suggested from population studies and although an establishment of cause-and-effect has

generally not yet been made they are topics of continuing study (Nutrition Reviews, 1991).

1.5.1.1.1 Fibre

Although it is accepted that dietary carbohydrate is generally absorbed and utilized by the body quite efficiently there is a portion of dietary carbohydrate which is unavailable. This unavailable carbohydrate together with lignin is what we currently refer to as dietary fibre (Jenkins, 1988).

Originally dietary fibre was considered to be composed of those constituents of the plant cell wall that resist the enzymes of the human gastrointestinal tract (Topping, 1991). At this point in time the term was synonymous with roughage, suggesting the importance of fibre as a bulking agent. As such it has played an important clinical role such as in the treatments of constipation and diverticular disease. It is now realized that apart from the major nonsoluble fibre constituents of cellulose, hemicellulose, and lignin that there are also many soluble nonstarch polysaccharides (guar gum, pectin, alginates, etc.). Although nondigestible by human enzymes they may be fermented to some degree by the microflora of the large bowel. Such fermentations may produce short chain fatty acids which are absorbable and thus could contribute some energy to the individual in question (Topping, 1991). The extent of this contribution has not yet been established. The soluble fibre constituents may also have some clinical value and Topping claims that they have the potential to decrease plasma cholesterol (Topping, 1991).

Although dietary fibre can be beneficial some possible adverse effects have also

been associated with it. Certain high fibre diets have been connected with such problems as sigmoid volvulus, esophageal cancer, and zinc deficiency (Jenkins, 1988). There are studies which suggest that the consumption of purified fibre preparations may induce negative zinc, magnesium, and calcium balance (Jenkins, 1988; Kelsay, 1986). With the consumption of whole foods fibre-associated substances such as phytate (inositol hexaphosphoric acid) may also contribute to metal binding.

1.5.1.2 Vitamins

Of the 13 vitamins which must be provided by the diet of man, four are fat soluble and the remaining nine are water soluble. The vitamins differ from one another not only in physicochemical structure but also in function (Wardlaw and Insel, 1990; Guthrie, 1989; Hunt and Groff, 1990).

The function of no vitamin is entirely understood yet but some physiological roles have been elucidated for each one. For example, vitamin A has been shown to be necessary for vision in dim light and the maintenance of a healthy epithelium among other things. Vitamin D once activated in the body, is extremely important to calcium and phosphorus metabolism and consequently is indirectly important to the maintenance of the skeleton. Vitamin E functions largely as a fat soluble antioxidant while vitamin K is essential for blood clotting. The water soluble B complex of vitamins (thiamin, riboflavin, niacin, pantothenic acid, biotin, pyridoxine, folate, and vitamin B₁₂) each portray various functions but many of these are roles played by the vitamins as coenzymes in assisting enzymes to catalyze various metabolic reactions. Vitamin C, also

has many known functions which include a coenzyme role and an antioxidant role (Hunt and Groff, 1990; Wardlaw and Insel, 1990; Guthrie, 1989; Carethers, 1988; Whitney and Hamilton, 1987; Munro, Suter and Russell, 1987; Marcus and Freedman, 1985; Grasbeck, 1984; Rosenberg et al. 1982; Nauss and Newberne, 1981; Gross and Newberne, 1980).

It has been suggested that certain nutrients, including some of the vitamins, may be necessary for immune functioning. There is evidence to suggest that thiamin, riboflavin, pyridoxine, pantothenate, biotin, folacin, vitamin B₁₂, ascorbic acid, vitamin A, and vitamin E are probably all required to support a normal immune response (Beisel, 1982; Axelrod, 1980). There is little to suggest that vitamins D and K have any significant role to play in immune function (Beisel, 1982).

1.5.1.3 Minerals

The body contains numerous minerals but only some of these are believed to be necessary for survival. Of the 22 which most agree should be provided by the diet, seven are found in the body in relatively large amounts and these tend to be referred to as the macronutrient elements (calcium, phosphorus, potassium, sulfur, sodium, chlorine, and magnesium). The remaining 15 are found in the body in much smaller quantities and have been labelled the micronutrient elements or the trace elements (iron, zinc, selenium, manganese, copper, iodine, molybdenum, cobalt, chromium, fluorine, silicon, vanadium, nickel, arsenic, and boron) (Guthrie, 1989). Other minerals probably also have metabolic importance but whether or not they are essential to life is not well established.

As for the vitamins, some functions of the minerals have been established and suggest that the minerals are a very varied group. Some are important constituents of the bone (calcium, phosphorus, and magnesium) while others play an electrolyte role (sodium, potassium, and chlorine). Even those found in the body in trace amounts can be of utmost importance. Iron and zinc, for example, each have numerous essential roles in the human body. Iron has been shown to assist in the bodily transport of oxygen and carbon dioxide, is a constituent of various enzymes, and is essential for erythropoiesis. Zinc is required as a cofactor by more than 200 enzymes. These enzymes catalyze various reactions and participate in such essential processes as protein and amino acid metabolism, chylomicron formation, nucleic acid synthesis, and expression of the genome (Guthrie, 1989; Wardlaw and Insel, 1990; Whitney and Hamilton, 1987; Hunt and Groff, 1990; Prasad, 1983; Mertz, 1981).

Some of the minerals have been shown to play an important role in normal immune functioning. Magnesium (Beisel, 1982; McCoy and Kenney, 1975) and selenium (Beisel, 1982; Sheffy and Schultz, 1979) have both been implicated but there is much more evidence for the importance of iron (Dallman, 1987; Beisel, 1982) and especially zinc (Prasad, 1983; Beisel, 1982; Duchateau et al. 1981; Loria, Hershko and Konijn, 1979).

1.5.1.4 Nutrient/Nutrient Interactions

Although distinct functions have now been elucidated for many of the nutrients much data suggests that these compounds are highly interreactive. This is a complex area

of nutritional science where much is still unknown.

There are numerous examples of nutrients which have been shown to react with one another and these relationships can be both positive and negative. For example, dietary fiber and phytate have been shown to have a negative effect on the body's retention of a number of minerals. Sandstead claims that it is phytate which is primarily responsible for the decreased calcium retention and zinc absorption which accompanies a high fiber diet (Sandstead et al. 1990). He also comments that zinc retention can be reduced by a high consumption of calcium, milk protein, and possibly also folate and ferrous iron supplements. Zinc and copper also interfere with the absorption of each other via the intestinal absorptive cells (Wardlaw and Insel, 1990).

Although many nutrients, especially minerals, appear to reduce the bioavailability of various dietary constituents there are also many positive nutrient/nutrient interactions. Vitamin C can exert a positive influence on the body's nutritional status by enhancing the absorption of iron and possibly calcium. Being an antioxidant it also helps to maintain folate in its reduced biologically active form. Also zinc absorption can be facilitated by digestible dietary proteins especially those with a high proportion of the amino acids histidine and cysteine (Sandstead 1991; Guthrie 1989; Spencer et al. 1987; Morley 1986; Sandstead 1982).

These examples illustrate only a few of the reported interactions between nutrients. Apart from interacting with each other they have also been shown to interfere with the metabolism of many drugs.

1.5.2 Nutritional Status of the Senior Adult

The general status of health in the elderly is lower than for the younger adult. Since nutrition is such an important aspect of general health it would be expected that the elderly also have a poorer nutritional status than the younger adult. Although research on the nutritional status of the elderly specifically is more limited than such work on other age groups, that which is available does support this expectation (Yeung et al. 1986; Eckholm, 1985; Rivlin, 1982; Exton-Smith, 1975).

Chandra estimates that 30-35% of all elderly experience malnutrition and that many of these experience deficiencies of two or more nutrients simultaneously (Chandra, 1990). The very old seem to be at a higher risk of malnutrition with nutrient deficiencies being twice as common in those over 80 years as compared to the younger elderly (Davies, 1990; Roebothan and Chandra, 1991; Czajka-Narins et al. 1987).

Malnutrition seems to be a problem whether these seniors are institutionalized or not (Baker et al. 1979). Some studies of institutionalized groups in the United States suggest that malnutrition is more common in those elderly individuals residing in institutions (Sahyoun et al. 1988; Munro, Suter and Russell, 1987) and in one nursing home tested residents showed a 52% incidence of malnutrition (Pinchcofsky-Devin and Kaminski, 1987). Other studies claim a high rate of malnutrition in the noninstitutionalized (Yearick, Wang and Pias, 1980; O'Hanlon and Kohrs, 1978). Indeed one study performed in Saskatoon suggests that the institutionalized elderly consumed diets somewhat superior to those living in private homes (Lee, Olson and Friel, 1984).

There is some controversy as to whether one's requirements for various nutrients change with age (Young, 1990; Suter and Russell, 1987; Morley, 1986; Marcus and Freedman, 1985). It is accepted that energy requirement is lower in later years (Health and Welfare Canada, 1990; Munro, Suter and Russell, 1987). Consequently the aged need to consume a smaller amount of food than they did in earlier years. If the requirements of many nutrients apart from energy are maintained or increased in later years, then the smaller volume of food would still have to supply adequate amounts of all nutrients and so the diet would need to be more nutrient dense. Such a nutrient dense diet may be difficult to plan and therefore could put the elderly at a higher risk of developing malnutrition. This may be an underlying cause for some of the nutritional problems of the elderly yet the types and causes of nutritional problems in the elderly are numerous and complex.

Today we must address the issue of overnutrition as well as undernutrition. Overnutrition applies not only to an excessive intake of energy but also to an excessive intake of vitamins and minerals. It has been suggested that up to 66% of the American population is taking nutrient supplements (Whitney and Hamilton, 1987). Although nutrient toxicities are a real problem for some, including some elderly, many of the nutritional problems of this age group are due to nutritional deficiencies.

1.5.2.1 Obesity

As for any other age group, some elderly are overweight. On the basis of a review of nutritional studies conducted in the United States from 1957 - 1967 Kelsay

noted that for subjects over 50 years of age, 20% - 68% were overweight (Kelsay, 1969). The Nutrition Canada Survey suggested that 65.8% of the general male population 65 years and older were at a high risk of being overweight according to the ponderal index (height / cubic root weight) while 79.9% of the general female population was at a high risk according to the same criteria (Health and Welfare Canada, 1973). There is evidence to suggest that the energy intakes of adults diminish with age yet the drop in intake appears not to equal the drop in energy requirements (Munro, Suter and Russell, 1987).

It is generally accepted that with age lean body mass drops and there is an increase in the relative proportion of body fat (Morley, 1986; Allen, Anderson and Langham, 1960). According to the standard criterion of obesity, being more than 20% over ideal body mass (Morley, 1986), many of the elderly can also be considered obese. It has been estimated that 25% - 41% of elderly males and 25% - 54% of elderly females are obese (Rossman 1979). Such calculations often do not consider the loss of height experienced after physical maturity (Morley, 1986; Rossman, Trotter and Gleser, 1951) and so the elderly may even be somewhat more obese than most estimates suggest.

Of importance to a discussion of obesity in the elderly is the fact that although in certain disease states such as hypertension and type II diabetes mellitus where weight reduction would certainly be warranted, there is no clear cut indication for weight reduction (Morley, 1986). There may be a lesser degree of morbidity and mortality associated with obesity in the elderly, especially the healthy elderly (Andres, 1980; Fanestil and Barrows, 1965). Since most people associate obesity with a higher risk of

functional abnormality it could be argued that this term, as it is currently defined, may not be applicable to the elderly.

Apart from total body fat it is also important to consider the distribution of body fat. It has been suggested that an excessive amount of fat in the trunk region may be a better indicator of the presence of risk factors than weight per unit height (Shimokata, Tobin, Muller, Elahi, Coon and Andres, 1989; Health and Welfare Canada, 1988). The waist:hip ratio (WHR) has been used as an indicator of body fat distribution. Shimokata has used data from the Baltimore Longitudinal Study of Aging to show that WHR increases with age in both sexes (Shimokata, Andres, Coon, Elahi, Muller and Tobin 1989).

1.5.2.2 Protein/Calorie Malnutrition

Protein calorie malnutrition (PCM) does occur in the elderly. In St. John's, Newfoundland Chandra found that 21 of the 51 subjects tested (above 60 years of age) were protein - calorie malnourished (Chandra et al. 1982). A group of institutionalized elderly in the United States were assessed and 52% were shown to be experiencing PCM of various degrees (Pinchcofsky-Devin and Kaminski, 1987). Kelsay, as a consequence of reviewing numerous U.S. nutritional studies, claims that 10% - 20% are underweight (Kelsay, 1969).

The American Health and Nutrition Examination Survey (HANES) suggests that diets of the elderly are often low in energy value with 16% caucasians and 18% blacks consuming less than 1000 kilocalories per day (Morley, 1986). Although true energy

requirements for the elderly are as yet controversial (Munro, Suter and Russell 1987; Mitchell and Lipschitz, 1982) suggested intakes for elderly Canadians with moderate physical activities are at least 1700 kilocalories per day (Health and Welfare Canada, 1990). It is difficult to set accurate energy requirements for many reasons, one being the discrepancies between data collected on intakes and expenditures by the methods now in use (Mertz et al. 1991, Schoeller, 1990).

Protein requirements are also not established with certainty in the elderly (Young, 1990; Morley, 1986) nor are the standards used to assess protein status (Thompson, Robbins and Cooper, 1987; Schrijver, VanVeelan and Schreurs, 1985; Mitchell and Lipschitz, 1982; Dybkaer, Lauritzen and Krakauer, 1981) but indications are that dietary protein intakes of the senior adult may sometimes be less than adequate (Nutrition Canada Survey, 1973). Mean protein intakes for Canadian women above 65 years have been estimated at 0.78 g per kilogram of body weight (g/kg BW) in comparison to the present recommendation of 0.86 g/kg BW. Men of the same age consume 0.93 g/kg BW protein (Health and Welfare Canada, 1990).

Because of the way in which the body utilizes protein and energy supplies, it is difficult to distinguish which factor is ultimately responsible for precipitating the clinical manifestations of protein calorie malnutrition but in the elderly both are suspect.

1.5.2.3 Vitamin Deficiencies

With the nutrient dense diet needed to meet the specific nutritional requirements of the elderly, evidence of vitamin deficiencies in this age group should not be

unexpected.

Certain vitamins appear to be of poorer status in the elderly than do others. The types of deficiencies seen in the elderly may be different than those seen in younger individuals.

1.5.2.3.1 The Fat Soluble Vitamins

The fat soluble vitamins tend to be stored in the body. Any excesses in intake which are not immediately needed to meet requirements are stored and are available for use in times of short supply. Therefore it usually takes some months for the deficiency of a fat soluble vitamin to develop although this varies somewhat from vitamin to vitamin (Hunt and Groff, 1990).

Rarely are deficiencies of vitamins E and K seen in man at any age (Wardlaw and Insel, 1990; Guthrie, 1989; Whitney and Hamilton, 1987). Although there is potential for vitamin D deficiency in the elderly where many are housebound, there is little evidence available to suggest that the real incidence of deficiency of this vitamin is any higher than that seen in other age groups. Similarly, little data is available on the status of vitamin A in the elderly but that which is available suggests that although as high as 29% of the American elderly may consume less of this vitamin than is recommended, less than 10% actually display serum levels which are lower than normal (Kelsay, 1969). The Nutrition Canada Survey monitored intakes and circulating levels of vitamin A. It also sought for keratinization of both follicles and the bulbar conjunctiva of the eye as clinical signs of vitamin A deficiency but no such signs were detected at any age (Health

and Welfare Canada, 1973).

Watson has recently supplemented the diets of elderly subjects with beta-carotene, a vitamin A precursor (Watson et al. 1991). Although they did find that the supplement positively influenced certain aspects of the immune response, it was not accompanied by a change in the circulating retinol level. It was concluded that the immunomodulation was due to the carotenoid and did not function through retinol. Recent work suggests that the elderly are fairly tolerant to high intakes of vitamin A (Stauber et al. 1991).

1.5.2.3.2 The Water Soluble Vitamins

Storage of the water soluble vitamins in man is generally limited. The human body has much less of a capacity to store water solubles as compared to fat solubles and so deficiencies of the water soluble vitamins are much quicker to establish with the possible exception of vitamin B₁₂. Although the body stores of this water soluble vitamin are also very limited its requirement by the body is so low that even this small storage is suffice to meet requirements for some time (Hunt and Groff, 1990).

1.5.2.3.2.1 Vitamin C

Some attempts have been made at assessing ascorbic acid (vitamin C) status in the elderly. One study of an elderly group residing in an English nursing home showed that ascorbic acid levels in the white blood cell were significantly lower in the elderly as compared to younger adults (Munro, 1980; Andrews, Letcher and Brook, 1969). Other reports of decreased levels of vitamin C in the elderly have also been made (Burr,

Lennings and Millbank, 1982; Burr et al. 1974). Kelsay also claims that there is evidence to suggest that vitamin C intakes may be poor in some of the elderly, the risk of this being higher in the low income groups (Kelsay, 1969).

There is some dispute as to whether these dropping levels of vitamin C are of any true physiological significance (Bates et al. 1977). Garry suggests that even considering the depressed levels of ascorbic acid which have been documented in some elderly groups, only a small percentage are really at risk of developing clinical symptoms of hypovitaminosis C (Garry et al. 1982).

1.5.2.3.2.2 Folacin

The Nutrition Canada Survey made Canadians realize that folacin deficiency was a problem which could and did affect all groups, including the elderly (Nutrition Canada Survey, 1973). Since then a number of studies have verified the poor status of this nutrient in some elderly (Morley, 1986; Marcus and Freedman, 1985; Rosenberg et al. 1982; Baker et al. 1979) especially those in certain subgroups of the senior adult population such as females of the lower socioeconomic classes (Wagner et al. 1981).

Intakes of folacin by the elderly are quite variable (Munro, Suter and Russell 1987). A U.S. study on folacin intakes of the elderly residing in Albuquerque, New Mexico suggested that 70% of males and 84% of females were consuming less than 3/4 of the American Recommended Dietary Allowance (RDA) (Garry et al. 1982). The Nutrition Canada Survey suggested that the elderly consume less of this nutrient than younger adults. Elderly males were reported to consume 151 mcg. per day while elderly

females consumed even less at 130 mcg. per day (Rosenberg et al. 1982, Health and Welfare Canada 1973). Although some problems still exist as to what the true requirement of this nutrient and many others are in the elderly (Kergoat et al. 1987, Thompson, Robbins and Cooper, 1987; Schrijver, VanVeelan and Schreurs 1985; Mitchell and Lipschitz, 1982; Dybkaer, Lauritzen and Krakauer, 1981), the Canadian RNI is set at 205 - 220 mcg. per day for elderly males and 190 mcg. per day for elderly females, values which far exceed the average intakes seen by the Nutrition Canada Survey. Milne claims that 200 mcg per day is sufficient to maintain folate nutriture in healthy males contained in a metabolic unit (Milne et al. 1983) while 50-100 mcg is probably needed to support normal hematopoiesis (Sullivan and Herbert, 1964; Marcus and Freedman, 1985).

Although intakes of folacin are low in the elderly this does not necessarily imply that this group has functional impairment of this nutrient. Deficiencies of late stage are unlikely with few elderly portraying clinical signs. Subclinical deficiencies which might be expected to be accompanied by low circulating levels of the vitamin exist at a prevalence of no greater than 6% in the U.S. (Rosenberg et al. 1982) but probably somewhat higher in Canada (Nutrition Canada Survey, 1973). Sneath found that 10%-20% of general hospital admissions in the United Kingdom had low serum folate levels but that the incidence was higher for geriatric patients with mental symptoms (Sneath et al. 1973). Based on work done by Rosenberg in the United Kingdom, serum and red blood cell levels of folate associated with elevated risks of deficiency were established for the elderly (Rosenberg et al. 1982). High risks of deficiency for this vitamin occur

with serum folate values below 3.0 ng/ml and red cell folate below 140 ng/ml.

1.5.2.3.2.3 Cobalamin

A deficiency of vitamin B₁₂ (cobalamin) is not common in man and when it does occur it tends to be associated with a physiological abnormality (Hunt and Groff, 1990; Morley, 1986). Normally the body requires a glycoprotein referred to as intrinsic factor for the efficient absorption of cobalamin. In some individuals the synthesis of intrinsic factor becomes defective in middle to late life and it is a deficiency of intrinsic factor which often is the physiological abnormality which precipitates cobalamin deficiency. Since this defect is more common in the later years, vitamin B₁₂ deficiency is more common to the elderly than to younger aged groups (Guthrie, 1989; Carethers, 1988).

Some nutritional status surveys of the elderly have tested cobalamin and although some suggest that the level of this vitamin does not drop in the elderly (Morley, 1986) most studies suggest that it does (Carethers, 1988; Baker et al. 1979). Some of the uncertainty may reflect the assays of vitamin B₁₂ which are available for present use (Morley, 1986). Herbert and Colman admit that there is no "gold standard" method presently available for the assay of this vitamin but that radioassay would be the method of choice (Herbert and Colman, 1988).

Carethers suggests a prevalence of vitamin B₁₂ deficiency in those above 65 years to be approximately 3% - 10% (Carethers, 1988).

1.5.2.3.2.4 Other Vitamins of the B Complex

Malnutrition in the elderly regarding other water soluble vitamins has been occasionally reported in the literature. The prevalence of these problems is likely low in our society and may not be significantly different than what is reported for younger individuals (Gary, Goodwin and Hunt, 1982). Kelsay's review of numerous American nutritional studies revealed that there was evidence to suggest a moderate risk of malnutrition in the elderly with regards to thiamin, riboflavin, and niacin (Kelsay, 1969).

Niacin intakes were found to be low in 57% of the elderly tested in Boston, 37% of these subjects were also deemed to be consuming inadequate amounts of riboflavin (Kelsay, 1969; Davidson et al. 1962). A smaller percentage of elderly were excreting low levels of urinary riboflavin in Syracuse and New York suggesting low body stores of this nutrient (Dibble et al. 1967; Brin et al. 1965; Brin-Schwartzberg and Arthur-Davies, 1964).

Iber suggests that most Americans consume adequate amounts of thiamin but that some of the elderly have a poor thiamin status (Iber et al. 1982). The elderly in Syracuse and New York were assessed for thiamin status. 18% - 50%, depending upon the specific study, showed signs of low urinary thiamin (Dibble et al. 1967; Brin et al. 1965; Brin, Schwartzberg and Arthur-Davies, 1964). Approximately 10% of those tested in New York also showed evidence of depressed transketolase activity suggesting that the levels of thiamin were low enough to disrupt normal biochemical functioning.

1.5.2.4 Mineral Deficiencies

Although the nutritional status of most minerals in the elderly has not drawn a lot of attention there has been some recent work done with calcium, iron, and zinc because of the important roles these minerals play.

1.5.2.4.1 Calcium

Osteoporosis is a condition found most often among middle-aged and elderly women. It is characterized by a reduced bone mass, high susceptibility to bone fracture, and bone pain. It has been estimated that the incidence of this condition in the elderly may be as high as 50%. A drop in bone density with age has been noted by many (Lee, Johnson and Lawlor, 1981). The condition is multicausal in origin but long term inadequate calcium intake is probably an important causative factor (Hunt and Groff, 1990; Munro, Suter and Russell, 1987; Morley, 1986).

Although the consumption of calcium rich foods such as milk and dairy products is important to the elderly, many studies suggest that it is well below recommended levels (Morley, 1986; Bowman and Rosenberg, 1982; Gary et al. 1982; National Dairy Council, 1982; Kelsay, 1969; LeBovit, 1965; Steinkamp, Cohen and Walsh, 1965; Fry, Fox and Linkswiler, 1963; Davidson et al. 1962). Heaney noted that the efficiency of calcium absorption can vary considerably according to what is consumed with it and so bioavailability must also be considered (Heaney, Weaver and Fitzsimmons, 1991).

Aggravating the low dietary intakes of calcium may be the overall dietary pattern of the elderly. They tend to consume limited amounts of calcium-rich foods but the other

components of their diets could exert negative influences on the calcium which is consumed. For example the elderly may substitute some tough meats for easier chewed plant foods. The latter tend to have higher levels of fiber and phytate which could bind up the calcium which is consumed and make it less bioavailable. Also high consumptions of phosphorus and protein which are typical of the North American diet can negatively influence one's calcium status (Hegsted, 1990; Avioli, 1988).

1.5.2.4.2 Iron

There is some controversy as to the extent of iron deficiency which is experienced by the elderly. Hallberg suggests that healthy elderly can maintain a good iron balance and therefore should be at low risk of developing nutritional iron deficiency anemia (Hallberg, 1983). Munro, Suter, and Russell agree that although some iron deficiency is seen in the elderly that the prevalence is low and suggest that this may be so because of good body stores of iron resulting from a lifetime accumulation of this mineral in males and accumulations made after 50 years in females (Munro, Suter and Russell, 1987; Lynch et al. 1982; Cook, Finch and Smith, 1976).

Although there is evidence suggesting good iron status in the elderly generally, there are conflicting findings on the dietary consumption of iron in this age group. While some report the mean iron intake of the elderly to be adequate (Lynch et al. 1982) some older studies have observed it to be low (Health and Welfare Canada, 1973; Fry, Fox and Linkswiler, 1963; Davidson et al. 1962). Apart from poor intakes, other possible contributors to poor iron status could be accelerated body losses and/or low

bioavailability of the mineral from the specific foods and food combinations consumed (Fairbanks and Beutler, 1988). In the elderly Herbert feels that it is the loss of blood, often from the gastrointestinal tract, which is the major cause of iron deficiency (Herbert, 1990). Low bioavailability of the mineral could also contribute to iron deficiency in the elderly since the availability of iron from meats is much higher than that from cereal products yet as one ages one tends to eat more of the latter than the former. The defective absorption of iron from diets high in cereal products and low in animal protein is well established (Fairbanks and Beutler, 1988).

Various tests are in use to assess the nutritional status of iron. Haematocrit values have been shown to be low in some elderly, especially females (Dibble et al. 1967; Brin et al. 1965). Both haematocrit and haemoglobin values were reported to drop with progressing age in a group of elderly Kentucky residents (Lee, Johnson and Lawlor, 1981). Morley reported a drop in haemoglobin values in males over 60 years but claims that they are maintained in females up to 84 years (Morley, 1986).

Probably a more reliable indicator of iron status is serum ferritin. Serum ferritin values in the elderly appear to be quite variable but there are reports of these values actually increasing with old age (Garry, Goodwin and Hunt, 1983; Loria, Hershko and Konijn 1979; Cook, Finch and Smith, 1976; Leyland, Harris and Brown, 1970). Ferritin is an acute phase protein and as such its circulating level rises with an infection. This is a possible explanation for the elevated serum values sometimes noted in the elderly.

Other methods of determining iron status are used. These include such measurements as total serum iron, circulating protoporphyrin levels (precursors to

haemoglobin synthesis), and percent saturation of transferrin. In early iron deficiency these values are often normal but the serum ferritin concentration is diminished (Fairbanks and Beutler, 1988).

Iron status may not be as bad in the elderly as was once believed. It should be realized that the anemia which has been reported in the elderly may be caused by factors other than iron deficiency. Indeed the poor haemoglobin and haematocrit values seen in some elderly may also have an alternate cause (Munro, Suter and Russell, 1987; Lynch et al. 1982).

1.5.2.4.3 Zinc

The past few years have supported an active interest in zinc by many researchers. It is now known to play a number of very important metabolic roles and physiological abnormalities associated with its deficiency in man are now being elucidated (Klevay, Reck and Barcome, 1979; Oleske et al. 1979; Stutman, 1974; Julius et al. 1973; Swenerton and Hurley, 1968). Zinc deficiency in the elderly is quite real with the risk of developing zinc deficiency being higher in the elderly of the lower socioeconomic classes (Sandstead et al. 1982; Wagner et al. 1981).

Zinc intakes are quite variable in the elderly (Abdulla et al. 1972). Many reports claim that these intakes are less than optimal (Munro, Suter and Russell, 1987; Nordstrom, 1982; Borgstrom et al. 1979; Gregor, 1977) and some question the availability of the zinc once it is consumed (Sandstead et al. 1982). It has also been suggested by one study that housebound elderly are in negative zinc balance (Bunker et

al. 1987).

There is much criticism concerning the tests commonly being used for the assessment of zinc status (Kergoat et al. 1987; Munro, Suter and Russell 1987; King, 1986). Although serum/plasma zinc values are questioned by some, they have been recorded for the elderly in a number of studies. Many of these suggest that serum zinc drops with progressing age (Morley, 1986; Bunker et al. 1984; Busher et al. 1982; Sandstead et al. 1982; Lindeman, Clarke and Colmore, 1971) but others suggest that it is maintained (U.S. Department of Health, Education, and Welfare, 1981). It has been claimed that hair levels of zinc in the elderly are low (Sandstead et al. 1982) but that red blood cell (RBC) zinc is probably maintained (Munro, Suter and Russell, 1987).

Many still question the true sensitivity of circulating zinc as an indicator of body status and Prasad suggests that a much more meaningful measurement would be the assessment of zinc levels in the lymphocytes, granulocytes, and platelets. He claims that such values can and have been successfully used as a more sensitive measure of body zinc. The thymic hormone thymulin requires zinc to function and it has also been suggested that monitoring the function of this hormone could also have some potential applicability (Prasad, 1988).

1.5.2.5 Aetiology

It has often been suggested that to study the cause of any disease state one must consider not only the suspected agent(s) but also the host itself and all of the other components of its environment. The cause of a nutritional deficiency is just as difficult

to find. Do observed nutritional deficits represent a dietary lack or a physiological dysfunction (poor digestion, absorption, or utilization of nutrients)? In many cases the cause is probably multiple (Jacob, Russell, Sandstead, 1985; Bowman and Rosenberg, 1982).

An inadequate dietary intake is probably an important factor (Suter and Russell, 1987; Munro, 1981). Causes for inappropriate eating practices are numerous and varied in themselves. These include psychosocial factors such as loneliness and bereavement of lost spouse or friend. Lack of the appropriate health education and financial restrictions can also be important (Yeung et al. 1986; Lee, Johnson and Lawlor, 1981).

Not only is the amount of the nutrient consumed of importance but also its dietary source. For example, many nutrients are less available from plant foods than animal foods (Hunt and Groff, 1990; Sandstead et al. 1982; Ismail-Beigi, Faraji and Reinhold, 1977). Also what mix of nutrients, foods, and drugs are eaten together determines the true biological contribution of each of the dietary constituents. Both nutrient/nutrient and drug/nutrient interactions can be important (Davies, 1990; Morley, 1986; Vitale and Santos, 1985). Drug usage and even dependency is quite high in the elderly (McGandy et al. 1986; Schneider et al. 1986; Rivlin, 1982).

The degenerative diseases which accompany aging have nutritional side effects but also the normal physiological changes of aging may contribute to malnutrition. Loss of teeth, loss of taste acuity, decreased acidity in the stomach, and/or impaired absorption all may negatively influence nutritional status (Yeung et al. 1986).

It is difficult to establish a cause for the high rate of malnutrition seen in the

elderly. If it is not due to an impaired intake or uptake then impaired metabolism and/or an increased requirement of the nutrient(s) in question could be important (Brown, 1990; Czajka-Narins et al. 1987; Munro, Suter and Russell, 1987; Morley, 1986; Yeung et al. 1986; Exton-Smith, 1982; Rosenberg et al. 1982).

1.6 NUTRITION AND IMMUNE FUNCTION

Over the past few decades it has become increasingly evident that proper nutrition is necessary to support an optimal immune response. What evidence is there that the malnourished have an altered immune response?

1.6.1 Specific Aspects of Immune Function Affected by Malnutrition

Although the story is quite complicated, malnutrition does appear to have a direct, significant, and negative effect on immune function. What specific aspects of immune functioning have been shown to be affected by malnutrition?

Obviously research in this area is influenced by numerous extraneous factors. The use of human subjects would complicate the picture even more (Keusch, 1981). This is why much of the recent work relating nutrition to immune function is done with animals and/or in the laboratory (Corman, 1985; Gross and Newberne, 1980; Edelman, 1977). Clinical trials are limited and most of these study children rather than adults but findings from children appear to be similar to those from adults (Gross and Newberne, 1980). From all areas of research it holds true that the degree of malnutrition parallels the degree of immunological impairment (Edelman, 1977).

1.6.1.1 Effects on the Adaptive Cellular Response

Such a complex interplay exists between T and B cells that it is very unlikely that malnutrition could affect one without the other yet cell mediated immunity appears to be the most severely affected as a consequence of malnutrition (Linn, 1987; Gross and Newberne, 1980; Watson and McMurray, 1979).

1.6.1.1.1 Lymphoid Organs

Atrophy of the lymphoid tissue has been noted both in malnourished animals and humans (Chandra and Wadhwa, 1989; Dourov, 1986; Gross and Newberne, 1980; Scrimshaw, Taylor and Gordon, 1990). Indeed as early as 1845 Simon referred to the thymus as a very delicate barometer of nutrition for it responded to malnutrition more quickly than the other organs (Simon, 1845).

Other lymphoid organs and tissues are negatively affected by malnutrition apart from the thymus. With malnutrition, both the spleen and the lymph nodes decrease in size and weight and display a change in architecture and cellular compartments (Burritt and Anderson, 1984; Dionigi, 1982; Chandra, 1981b). A drop in tonsil size has also been noted (Chandra, 1983a; Chandra, 1972) and Chandra has discussed the atrophy of gut associated lymphoid aggregates seen in autopsies of children dying with severe PCM (Chandra, 1983a). Follis first observed the deterioration of many of these tissues in the malnourished rat more than fifty years ago (Follis, Day and McCollum, 1941).

1.6.1.1.2 Thymic Hormone

A depressed thymic hormone activity has been noted to accompany malnutrition (Chandra and Wadhwa, 1989; Chandra, 1981b; Chandra, 1980b). This may be part of the reason for the elevated proportion of "null" cells seen in the malnourished (Dionigi, 1982; Chandra, 1981b; Chandra, 1980b; Chandra, 1979a). These lymphocytes have neither B or T cell markers and are probably immature.

1.6.1.1.3 T Cells

A drop in the total number of circulating lymphocytes with malnutrition has been noted by some researchers (Edelman, 1977; Chandra, 1972). Others claim that lymphopenia is experienced by only a portion of the malnourished (Corman, 1985; Chandra, 1981b). Chandra suggests that approximately 15% of the malnourished display lymphopenia (Chandra, 1981b). The drop in total lymphocyte number could reflect a drop in T cell number.

1.6.1.1.3.1 T Cell Number

A decrease in the number of peripheral blood T cells has been noted in some but not all of the malnourished (Dourov, 1986; Chandra, 1983a; Keusch, 1981; Schlesinger, Munoz and Heresi, 1981; Chandra, 1980b; Gross and Newberne, 1980). A drop in the number of intraepithelial T lymphocytes has also been observed (Chandra, 1981b).

Little has been published concerning a possible redistribution of T cell subsets although Chandra suggests that there could be a drop in the ratio of T₄ helper cells to T₈

suppressor cells (Chandra, 1983b) while Shek, Waltenbaugh, and Coons claim that there may be a drop in T_g suppressor cells (Shek, Waltenbaugh and Coons, 1978).

1.6.1.1.3.2 T Cell Function

There is some indication that T cell function may also be affected by malnutrition. The *in vitro* response of lymphocytes to various mitogens is depressed (Chandra, 1983a; Dionigi, 1982; Schlesinger, Munoz and Heresi, 1981; Gross and Newberne, 1980; Chandra, 1974; Coovadia et al. 1974) but there is some variability in results (Chandra, 1981b). A subnormal response to the administration of both recall and new antigens in the delayed cutaneous hypersensitivity test has been observed in both rats and man (Chandra and Wadliwa, 1989; Dourov, 1986; Nohr et al. 1986; Corman, 1985; Chandra, 1981b; Schlesinger, Munoz and Heresi, 1981; Chandra, 1980b; Gross and Newberne 1980; Edelman, 1977; Chandra, 1972; Lloyd, 1968). There appears to be a drop in both induration diameter and percent responders (Burritt and Anderson, 1984). Edelman is not convinced that poor DCH is due to malnutrition *per se* and not the infection which is experienced by most of the malnourished (Edelman, 1977). It has been well documented that both viral and bacterial infections can induce a state of relative unresponsiveness to the test (Coovadia et al. 1974; Heiss and Palmer, 1974; Schlesinger and Stekel, 1974; Siasoco, Chen and Chang, 1974; Reed, Olds and Kisch, 1972).

Lymphokine production may also be affected (Schlesinger, Munoz and Heresi, 1981). There is some evidence that the production and/or release of macrophage migration inhibition factor and leukocyte migration inhibition factor may be subnormal

in states of malnutrition yet others claim that they are unaffected (Chandra, 1981b). Interferon production may be low or even absent (Schlesinger, Munoz and Heresi, 1981; Gross and Newberne, 1980). Also the synthesis of interleukin 1 may be depressed (Chandra and Wadhwa, 1989). Hoffman-Goetz, Keir, and Young suggest that not only is the synthesis of IL-2 depressed but so is the ability of T lymphocytes to respond to this cytokine (Hoffman-Goetz, Keir and Young, 1986).

1.6.1.2 Effects on the Adaptive Humoral Response

The results of studies on humoral immunity and PCM are both conflicting and complex (Gross and Newberne, 1980). The response to antigens seems variable but there is probably a drop in the primary antibody response to most antigens, especially where the response requires T and B cell co-operation (Dionigi, 1982; Chandra, 1981a). The affinity of the antibody for the antigen may also drop somewhat (Chandra and Wadhwa, 1989; Schlesinger, Munoz and Heresi, 1981; Reinhardt and Steward, 1979; Passwell, Steward and Soothill, 1974) and an increase in antibody-antigen complexes has been noted in some of the malnourished (Chandra and Wadhwa, 1989).

Malnutrition appears to have its major humoral effect on secretory IgA. Its concentration is reduced in mucosal secretions, tears, and saliva (Corman, 1985; Chandra, 1983a; Chandra, 1983b; Schlesinger, Munoz and Heresi, 1981). Although the B cell number appears largely unaffected there may be a selective drop in sIgA-bearing cells (Chandra, 1983a; Schlesinger, Munoz and Heresi, 1981) and a rise in IgM-bearing cells (Chandra, 1983a).

1.6.1.3 Effects on Accessory Factors/Cells

The number of phagocytic cells is probably maintained in a malnourished state but there is some suggestion that they have an altered functional capacity (Corman, 1985; Chandra, 1981b). Chemotaxis and engulfment by the PMNL may or may not be normal (Santos, Arredondo and Vitale, 1983) yet it has been suggested that their bactericidal and fungicidal activities may be compromised (Chandra and Wadhwa, 1989; Schlesinger, Munoz and Heresi, 1981; Gross and Newberne, 1980). The phagocyte could travel to the antigen and engulf it as normal but yet have a lesser ability to kill it intracellularly. Some evidence supporting an altered functioning of the neutrophil with malnutrition include a possible impairment of the glycolytic pathway (Gross and Newberne, 1980), a claim that the hexose monophosphate shunt is not elevated in response to phagocytosis as it normally is (Chandra, 1981a), and a low lysozyme content (Chandra et al. 1977). Lysozyme levels in the plasma and some body secretions have also been reported to be reduced in malnutrition (Chandra, 1981b; Schlesinger, Munoz and Heresi, 1981).

It has been claimed by a number of researchers that the level of many complement components, possibly all but C4, drops with malnutrition (Chandra and Wadhwa, 1989; Arvieux, Yssel and Colomb, 1988; Corman, 1985; Chandra, 1981b; Schlesinger, Munoz and Heresi, 1981; Gross and Newberne, 1980; Klein et al. 1977; Sirisinha et al. 1977; Kulapongs, 1976). This would explain the drop in total hemolytic activity seen. Schlesinger, Munoz, and Heresi suggest that a drop in complement could be a consequence of reduced protein synthesis and/or an increased catabolism of the complement components (Schlesinger, Munoz and Heresi, 1981).

1.6.2 The Third World

Early interest in the interrelationship of immune function and nutrition was sparked by field studies conducted on protein-calorie malnourished children in various countries of the world suggesting that their immunocompetence was negatively affected, often dramatically so (Scrimshaw, Taylor and Gordon 1990; Good and Lorenz, 1988; Neuman et al. 1975). Children deprived of an adequate protein-calorie intake early in life were often characterized by gross deficits in antibody production and severe cell mediated immune dysfunction (Good and Lorenz, 1988; Chandra, 1981c; Koster, Gaffar and Jackson, 1981; Neuman et al. 1977; Schlesinger et al. 1977). Low serum levels of thymic hormone were also noted (Chandra, 1979a). The immunodeficiency was found to be largely reversible in many instances with nutritional therapy (Burritt and Anderson, 1984; McMurray, Watson and Reyes, 1981; Chandra, 1979b) yet sometimes the immunodeficiency was seen to persist (Good and Lorenz, 1988; McMurray, Watson and Reyes, 1981) and occasionally it was suggested that protein-calorie malnutrition (PCM) could even boost some immune functions (Good and Lorenz, 1988). Obviously the complexity of the malnutrition immune function interrelationship is considerable.

PCM is a major nutrition problem in the world today (Santos, Arredondo and Vitale, 1983). With such a profound influence on the functioning of the immune system it must also be a major contributor to immune dysfunction and indeed Burritt and Anderson suggest that "Malnutrition is the most frequent cause of secondary immunodeficiency world-wide" (Burritt and Anderson, 1984)³. It is a condition which

³ Burritt MF, Anderson CF. Laboratory Assessment of Nutritional Status. Human Pathology 1984; 15:131.

is experienced by large numbers in the industrialized world as well as the third world (Burritt and Anderson, 1984; Linn and Jensen, 1984; Bistran et al. 1976; Bistran et al. 1975; Law, Dudrick and Abdou, 1973).

1.6.2.1 Complications of Studying PCM and Immune Function

These field studies on PCM children are certainly informative and important due to the widespread nature of the disease and they form the foundation for our understanding of the nutrition immunity link yet they are often difficult to interpret. There are so many factors influencing these children that it is difficult to say that nutrition is at all responsible for the immune dysfunctions observed. Even if nutrition is a causative factor, as subsequent research has suggested, it is still difficult to sort out what aspects of the immune problem are caused by what aspects of the nutritional deficit. It is not practical to group all PCM children together. They constitute a very heterogeneous group. They differ in the cause of the disease, accompanying nutritional deficits, accompanying infection, the time of onset, the severity and duration of the insult, and other such factors, all with considerable impact on the functioning of the immune system. Even the different environmental exposures and genetic makeups of such human subjects makes their study somewhat complicated (Cunningham-Rundles, 1982).

1.6.2.1.1 Aetiology of PCM

It is very difficult to distinguish protein malnutrition from energy malnutrition (a caloric deficit) in man. The human body treats energy as a top priority. If an

individual is consuming a diet which is adequate in protein but is deficient in calories then the body will use protein in an attempt to meet its caloric requirements. Consequently although the individual in question is consuming an adequate diet in regards to protein the body may still lack an adequate protein supply since much of it was redirected and used to meet energy requirements rather than protein requirements.

In an attempt to recognize the interrelationship of these two conditions, they are generally considered together as protein-calorie (PCM) or protein-energy malnutrition (PEM). PCM thus represents a continuum of disease states with marasmus at one end of the continuum and kwashiorkor at the other. Marasmus accompanies a diet lacking in quantity - a diet of adequate composition yet deficient in total caloric content. Kwashiorkor accompanies the consumption of an adequate amount of food and thus an adequate energy supply but there is an inadequate supply of quality protein. Most people experiencing PCM fall towards the middle of the continuum (Caliendo, 1979).

There is evidence to suggest that where one sits upon the PCM continuum influences how one's immune system could be affected by this condition (McMurray, Watson and Reyes, 1981; Bistran et al. 1977; Good, 1977; Schlesinger and Stekel, 1974; Schonland, 1972). Sirisinha for example, claims that marasmus and kwashiorkor have different effects on the complement system (Sirisinha et al. 1977). It has also been suggested that marasmus has less of a negative effect on the immune system than kwashiorkor (Nutrition Reviews, 1978). Schlesinger, from his work with Chilean children, claims that marasmic children experience multiple nutritional deficiencies much less often than do children with kwashiorkor (Schlesinger et al. 1977). This could be one

reason for the fact that kwashiorkor may be more immunosuppressive.

PCM is classified according to intensity and duration as well as by the type of deficit (whether it be protein, energy, or both). Such a classification is important for both diagnosis and treatment.

The intensity of the disease is determined mainly by anthropometry since biochemical and clinical abnormalities are usually not apparent until the disease is well advanced. Classification of the disease as acute, chronic, or acute with a chronic background (duration) is also done primarily by anthropometry (Torun and Viteri, 1988). The only apparent physical abnormality in mild and moderate PCM is weight loss. A drop in subcutaneous fat may also become apparent.

Severe PCM is diagnosed by clinical features and diet history (Torun and Viteri, 1988). Marasmus is characterized by a weight/height value of no more than 60% of the expected. There is generalized muscle wasting and an absence of subcutaneous fat. The hair is sparse, thin, and dry and the skin thin, dry, and usually wrinkled. Constipation is common but diarrhea can occur. There is marked weakness, behavioural changes, and often drops in heart rate, blood pressure and/or body temperature. Infections usually accompany marasmus (Torun and Viteri, 1988; Guthrie 1989; Caliendo 1979).

The predominant distinguishing feature in kwashiorkor is pitting, painless edema usually in the feet and legs but sometimes it extends to the upper extremities and even the face. Most patients have skin lesions in the areas of edema. The epidermis peels easily, exposes underlying tissues, and thus promotes infection. Although there may be some muscle wasting it is not as severe as in marasmus and the edema often masks it.

The hair is dry and brittle. Behavioural changes, hepatosplenomegaly, and tachycardia are common. Muscle tone and strength are reduced. As for marasmus, diarrhea and infections often occur (Torun and Viteri, 1988; Guthrie, 1989; Caliendo, 1979).

1.6.2.1.2 Multiple Deficiencies

Nearly all people experiencing PCM suffer multiple nutritional deficiencies. This applies not only to children in the third world but also to those protein-calorie malnourished in our own society (Roebathan and Chandra, 1991). With recent research suggesting the importance of deficiencies of single nutrients on immune function (Chandra and Wadhwa, 1989; Beisel, 1982; Fraker and Leucke, 1981; Gross and Newberne, 1980), the presence of many of these deficiencies with PCM does complicate the picture. Golden et al. even suggest that these accessory nutritional deficiencies could have a more detrimental effect on immune function than PCM itself (Golden et al. 1978).

1.6.2.1.3 Infection

It has been noted that malnourished children of the third world experience a higher number of more severe infections than children of better nourished populations (Linn, 1987; Chandra, 1980c; Scrimshaw, Taylor and Gordon, 1969). This may be partly due to poor sanitation and personal hygiene (Chandra and Wadhwa, 1989) but it is also a consequence of their poor nutritional health.

The incidence of infection in some malnourished groups is very high and it can be very difficult to separate those who are experiencing an infection from those who are

not (Schlesinger et al. 1977). A contributing factor is that young children with PCM often do not experience fever. This hypothermia may be due to a high body surface area and low subcutaneous fat but may also reflect a depressed synthesis and release of endogenous pyrogen (Neumann, 1977). Although fever may or may not be present, malnourished children can maintain high serum IgG levels with infection (Keusch, 1982; Chandra, 1972). Beisel claims that PCM children are also able to produce normal levels of C reactive protein (Beisel, Cockerell and Janssen, 1977). Keusch has some doubt that the acute phase response is retained intact in very severe cases of PCM (Keusch, 1981).

In the third world the interaction between PCM and infection is important but complex. "It is not merely by chance that famine and pestilence are two of the dreaded "Four Horsemen of the Apocalypse". They might well have been depicted riding the same horse ..." (Watson, 1981)⁴. It is not uncommon to see a cycle of malnutrition, infection, severe malnutrition, and death. Malnutrition can lead to infection but infection can also precipitate malnutrition (Caliendo, 1979).

Parratt suggests that the body is constantly exposed to numerous potentially harmful microorganisms but that it maintains high titres of antibody to keep these in check. The immune system must function very efficiently to keep abreast of an expanding population of microorganisms (Parratt, 1980). Anything which disrupts the normal functioning of the immune system therefore has the potential to precipitate an infection.

The first line of defense against any invading microorganism is the physical barrier composed of the skin and mucous membranes. The integrity of these physical

⁴ Watson RR. Nutrition and Immunity. *Journal of Dentistry for Children* 1981;48(6):443.

barriers is dependent upon the availability of such nutrients as protein, vitamin A, vitamin C, vitamins of the B complex and zinc (Santos, Arredondo and Vitale, 1983; Neumann, 1977). Therefore a deficiency in any one of these could lower an individual's resistance to infection.

Malnutrition not only can increase the likelihood of microorganisms gaining access to the interior of the body but a vast amount of research suggests that malnutrition also contributes to a depressed immune response suggesting that infiltrating microorganisms also have a better chance to survive and multiply.

Infection itself can be the cause of malnutrition. An infection or a recurrent mild infection could precipitate PCM in a marginally deficient child. The state of infection is accompanied by a sequestration of certain minerals within body pools or depots (Vitale and Santos, 1985; Keusch, 1982; Gross and Newberne, 1980; Neumann, 1977) in an attempt by the body to limit access of the invading pathogens to these nutrients yet in so doing these trace minerals are also of limited functional availability to the host. Also infections have been accompanied by decreased levels of some B complex vitamins (Neumann, 1977) and negative nitrogen balance (Santos, Arredondo and Vitale, 1983).

1.6.2.1.4 Timing of the Insult

There is a number of animal studies which suggest that the time at which a protein and/or calorie deficiency is experienced influences the negative physiological outcome of that deficiency (Jose and Good, 1971; Jose and Good, 1973). The younger an animal is when it is protein-calorie malnourished the greater the chance that it will

have permanently stunted growth (Nutrition Reviews, 1980; Ramalingaswami, 1969; Winick and Noble, 1966). The effects of malnutrition at an early age could be longlasting (Beach, Gershwin and Hurley, 1982; Chandra, 1981b).

Similar data has come from human observations (Chandra, 1981a; Nauss and Newberne, 1981; Gross and Newberne, 1980; Edelman, 1977). The effects of malnutrition on host defense for example, are less severe in adults than in children and even within the very early years, fetal malnutrition can be worse than postnatal malnutrition (Fraker, Jardieu and Cook, 1987; Chandra, 1981b; Chandra, 1974).

The timing of the initial insult is important but so is the time span over which the insult was experienced. The longer the period of malnutrition, the more negative are its consequences (Dourov, 1986; Nauss and Newberne, 1981).

1.6.2.1.5 Severity of the Insult

Another important factor which complicates the relationship between malnutrition and immune function is that malnutrition is experienced at different severities by different individuals. More severe states of PCM are associated with more negative impacts on immune functioning (Schlesinger, Munoz and Heresi, 1981; Edelman, 1977). The severity of malnutrition has been shown to be correlated with the extent of anergy to a delayed cutaneous hypersensitivity skin test (Burritt and Anderson, 1984; Smith et al. 1977) and to thymic atrophy (Dourov, 1986).

Although severe states of malnutrition have the most devastating consequences most malnutrition, even in the third world, is not severe (McMurray, 1981; Edelman,

1977). There is much support for the fact that moderate and even marginal malnutrition also significantly influence functioning of the immune system (Chandra, 1981d; Good, 1981; McMurray, 1981).

1.6.2.1.6 Stress

The stress which a PCM body undergoes must be tremendous. People differ in their ability to cope with stress. Stress has been shown to interfere with the immune response (Sugawara et al. 1990). It has been suggested that the stress associated with malnutrition is the cause of depressed immune function and not the malnutrition itself.

The circulating levels of many hormones such as corticosteroids, insulin, epinephrine, and thyroxin can change with PCM (Payne-Robinson et al. 1990; Gross and Newberne, 1980). Some of these have immunosuppressive effects (Cunningham-Rundles, 1982; Mark, 1981; Gross and Newberne, 1980; Neumann et al. 1977; Bell, Halzell and Price, 1976).

Glucocorticoids are immunosuppressive and their levels have been shown to be higher in PCM children as compared to controls (Hegggers, 1979; Neumann, 1977; Neumann et al. 1977; Schonland, 1972; Beisel and Rappaport, 1969). Leonard and MacWilliam claim that the fraction of physiologically active unbound glucocorticoids in blood of the malnourished can exceed that of the well nourished by more than 200% (Leonard and McWilliam, 1964). This may be at least partially due to the lower levels of proteins such as albumin which are available to bind these hormones in PCM (Gross and Newberne, 1980; Neumann et al. 1977; Schonland et al. 1972). Klein suggests that

endotoxin may contribute to the elevation of some of these hormones (Klein et al. 1977; Wolff, 1973). Endotoxin could be associated with immune dysfunction in PCM and malnourished children may be especially sensitive to it.

Regardless of the cause of these hormonal changes they do appear to exist and they also seem to be reversible with feeding. Rao, Srikantia, and Gopalan have shown that in PCM children cortisol, the major glucocorticoid in man, returns to normal levels with four to five weeks of nutritional therapy (Rao, Srikantia and Gopalan, 1968). The question therefore arises as to whether the immune suppression which accompanies malnutrition is not directly caused by malnutrition but is instead due to hormonal changes which accompany malnutrition and whose levels are elevated due to the stresses of PCM.

One argument presented against this is that immune suppression accompanies malnutrition but elevated cortisol levels do not always accompany malnutrition. Paisley suggests that although many PCM children do show high levels of circulating cortisol that it is the additional stresses which accompany the malnutrition which are responsible for this rise and that some PCM patients do not experience this hormonal change (Paisley, Angers and Frenk, 1973).

Animal research also exists which suggests that although hormonal effects may suppress immune function in PCM that the malnutrition itself also has a direct and a significant effect. Findings on rats support Paisley's suggestion. Rats maintained on a zinc deficient diet for 21 consecutive days showed no change in corticosterone levels nor in response to two other stresses administered (Reeves, Frissell and O'Dell, 1977) yet zinc deficient rats have been shown to experience immunosuppression such as a

significant drop in the response of lymphocytes to mitogens (Nutrition Reviews, 1980; Gross et al. 1979).

Another argument to support a direct link between malnutrition and immune suppression is suggested by work on mice. Fraker and Leucke's work with the mouse has shown that a nutritional deficiency, in this case a zinc deficiency, is associated with high plasma corticosterone and reduced T helper cell function but that approximately 50% of the T cell malfunction occurred prior to corticosterone elevation (Fraker and Leucke, 1981). They claim that nutritional deficiency does impair immune function and that although the mechanism is not clearly understood that it is probably not through corticosterone.

1.6.3 Contributions of Specific Nutrients other than Protein and Energy

Although most of the research done on malnourished subjects has been done thus far on the protein/calorie malnourished, it is becoming more obvious that attention should also be focused on subjects with single nutritional deficiencies (Beisel, 1990). PCM is just one of many possible deficiencies which an animal or a human could experience and those experiencing this problem are influenced by so many complicating factors that the cause of any abnormalities detected can be difficult to interpret. Although there is difficulty in obtaining human subjects for many single nutrient deficiencies, there has been a recent research emphasis in this direction in an attempt to clarify the relationship between nutrition and immune function. "These studies of isolated vitamin deficiencies lead to the inescapable conclusion that a single nutrient deficiency can result in profound

impairment of specific immunologic processes - a concept that has not yet received widespread attention or general acceptance."(Gross and Newberne, 1980)⁵

Studying nutrient deficiencies in isolation of one another should clarify the specific roles of the nutrients in immune function yet it should always be remembered that in the real life situation these deficiencies often coexist. Even a deliberate attempt to develop a single deficiency may be unsuccessful. In regards to zinc for example, the deficiency once established impairs appetite and could lead to an inadequate dietary intake and subsequent additional nutritional deficiencies (Fraker, Jardieu and Cook, 1987).

Research not only singles out the nutrients but also studies their effects separately on distinct aspects of immune function such as a DCH skin test, an in vitro proliferative response to a mitogen, or a count of B cells. Again this makes interpretation of findings much easier but it must always be remembered that the immune response is highly integrated and complex and involves the simultaneous functioning of many processes. "...many components of the immune system have been studied frequently in isolation from other parameters and apparently out of context with other critical environmental and genetic variables which influence the clinical onset and course of many pathologic conditions."(Taylor et al. 1979)⁶

⁵ Gross RL, Newberne PM. Role of Nutrition in Immunologic Function. *Physiological Reviews* 1980; 60(1):260.

⁶ Taylor MA, Israel BA, Escobar MR, Berlinerman D. In Vitro and In Vivo Parameters of Humoral and Cellular Immunity in an Animal Model for Protein-Calorie Malnutrition. *Advances in Experimental Medicine and Biology* 1979; 121(A):599.

1.6.3.1 Minerals

It is this area of nutrition alone where more is known about the effects of minerals than vitamins. Although essentially nothing is known concerning the relationship of many minerals with immune function research on zinc and iron is beginning to contribute a lot to our knowledge of the role of individual nutrients in immune function.

1.6.3.1.1 Zinc

Due largely to recent work with zinc deficient rodents more is known about the effects of this deficiency on immune function than that of any other single nutrient (Castillo-Duran et al. 1987; Fraker, Jardieu and Cook, 1987; Dowd et al. 1986; Cunningham-Rundles, 1982; Dionigi, 1982; Keusch, 1982; Chandra, 1980b). There are no major body storage depots for zinc and so the deficiency can develop rather quickly and easily (Beisel, 1982).

An inherited genetic defect has told us a lot about the clinical manifestations of zinc deficiency. Acrodermatitis enteropathica in man and a similar condition in a variety of Friesian cattle (lethal trait A46) share the primary defect of an extremely limited capacity to absorb dietary zinc by the intestinal cells (Beisel, 1982; Nutrition Reviews, 1981). Low body zinc results accompanied by numerous physiological abnormalities including immunodeficiency and an enhanced susceptibility to infection.

As for PCM the timing of a zinc deficiency seems to be of utmost importance in determining how severe its effects will be both on immune function and otherwise. Fraker and Leucke claim that mice deprived of zinc even as neonates had their immune

problems reversed with zinc therapy (Fraker and Leucke, 1981). Most researchers however feel that if the deficiency was experienced in utero that the resultant immunodeficiency is persistent in the offspring (Fraker, Jardieu and Cook, 1987; Fraker et al. 1986). The severity of the zinc deficiency is likely also important.

Zinc is required for the functioning of more than 70 enzymes (Sandstead et al. 1982; Chandra, 1980a) including thymidine kinase, DNA polymerase, and RNA polymerase. Zinc directly regulates both DNA and RNA synthesis (Gross and Newberne, 1980; Anthony, Hsu and Iber, 1975; Hsu and Anthony, 1975). Cellular and humoral immune responses depend upon the ability of immunocytes to rapidly proliferate and therefore ultimately on DNA, RNA, and protein syntheses, all processes which require zinc (Dionigi, 1982).

Zinc functions optimally within a specific range. Not only is a deficiency of this mineral responsible for negative effects on immune functioning but also excessive body levels have been associated with membrane defects and consequential malfunctions in phagocytic cells (Chandra, 1984; Chandra, 1980a; Gross and Newberne, 1980) and the complement cascade (Montgomery, Chvapil and Zukoski, 1979).

1.6.3.1.1.1 Effects of Deficiency on Lymphoid Organs

In both animals and man zinc deficiency has been associated with atrophy of the lymphoid organs. Most attention has been given to thymic atrophy (Fraker, Jardieu and Cook, 1987; Fraker et al. 1986; Beisel, 1982; Fraker and Leucke, 1981; Chandra and Au, 1980; Good, West and Fernandes, 1980; Loria, Hershko and Konijn, 1979) but the

spleen, lymph nodes, Peyer's patches, and other intestinal lymphoid tissues are affected similarly (Beisel, 1982; Gross and Newberne, 1980; Nutrition Reviews, 1980). Horribin suggests that prostaglandin E_1 is of primary importance in the regulation of thymus development and that its production is dependent upon numerous nutritional factors including zinc (Horribin et al. 1979; Manku et al. 1979). This could help explain the thymic atrophy associated with zinc deficiency.

1.6.3.1.1.2 Effects of Deficiency on Hormonal Milieu

Dardenne suggests that a specific change in thymic endocrine function is seen with zinc deprivation (Dardenne et al. 1984). The production of thymulin, a thymic hormone, requires zinc as a cofactor (Fraker et al. 1986; Dardenne et al. 1982) and apothymulin levels increase with a zinc deficiency. Many researchers have noted a reduced thymic hormone activity in zinc deficient states (Prasad et al. 1988; Fraker, Jardieu and Cook, 1987; Beisel, 1982; Iwata et al. 1978). This may be partly responsible for the immature T cells seen in states of zinc deprivation (Nutrition Reviews, 1983).

1.6.3.1.1.3 Effects of Deficiency on the Adaptive Immune Response

Both the cellular and humoral arms of the adaptive immune response are affected by a zinc deficiency but the major problem is with the cellular response (King and Fraker 1991; Prasad et al. 1988; Fraker et al. 1986; Nutrition Reviews, 1983; Dionigi, 1982; Fraker and Leucke, 1981; Nutrition Reviews, 1980). There is some suggestion that the number of peripheral blood lymphocytes drops (Gross and Newberne, 1980). This could

be accompanied by a redistribution of lymphocyte subsets (Fraker et al. 1986) with an increased number of immature thymocytes (Nutrition Reviews, 1983) and a decreased number of functional T cells (Fraker, Jardieu and Cook, 1987; Beisel, 1982).

T cell function may also be impaired. Dowd suggests that the generation of IL-2 and the corresponding receptor are determined by intracellular zinc status (Dowd, Kelleher and Guillou, 1986). Numerous results to both delayed cutaneous hypersensitivity skin tests and in vitro tests of proliferative response to mitogens also suggest that T cell function could be adversely affected. DCH in zinc deficient mice, guinea pigs, and cattle improves significantly with zinc therapy (Nutrition Reviews, 1983; Beisel 1982; Fraker, Zwickl and Leucke, 1982). The incidence of anergy in the elderly and in those receiving formula feedings (IV or oral) is significantly associated with plasma zinc concentrations (Bogden et al. 1987; Nutrition Reviews, 1983; Pekarek et al. 1979). In a study of 10 malnourished children Golden administered *Candida* intradermally to both forearms of each child. Subsequently one forearm was covered with a zinc sulfate ointment and the other a placebo. Topical zinc significantly improved the DCH response to the tested antigen (Golden et al. 1978).

In vitro tests of immune cells from both zinc deficient animals and humans show that they have a poor proliferative response to mitogens but that this response is regained with zinc administration (Bogden et al. 1987; Nutrition Reviews, 1983; Beisel, 1982; Allen, Kay and McClain, 1981; Gross et al. 1979; Pekarek et al. 1979). This applies not only to PHA but also to mitogens which preferentially stimulate the proliferation of B cells. Some suggest that zinc itself could be a weak mitogen (Cunningham-Rundles,

1982; Gross and Newberne, 1980) and/or can augment the the response to mitogens in an adjuvant-like manner (Fraker et al. 1986).

The proliferative response of B cells may be subdued in zinc deficiency but there is some controversy as to whether the number of antibody producing cells is maintained or not (Fraker, Jardieu and Cook, 1987; Nutrition Reviews, 1983). Although very little work has been done in the area, there is some evidence to suggest that an abnormal serum profile of immunoglobulins may accompany a zinc deficiency in some animals (Nutrition Reviews, 1980).

1.6.3.1.1.4 Effects of Deficiency on the Innate Immune Response

Schloen claims that in man zinc status is associated with the evolution of or resistance to cancer (Schloen et al. 1979). Some animal work gives preliminary support to this concept. A low natural killer cell activity has been noted in some zinc deficient rodents (Fraker et al. 1986; Beisel, 1982; Chandra and Au, 1980). A low T lymphocyte killer activity has also been suggested (Nutrition Reviews, 1983; Fernandes et al. 1979). Zinc deficient mice inoculated with allogeneic tumour cells were unable to respond to this challenge with normal natural killer cell and T lymphocyte killer cell activities (Nutrition Reviews, 1983; Beisel, 1982; Fernandes, Nair and Onoe, 1979).

Zinc deficient mice may also display dysfunction of phagocytic cells. It has been suggested that granulocytes have impaired chemotaxis and phagocytosis (Nutrition Reviews, 1983).

1.6.3.1.2 Iron

An iron deficiency is one of the most likely forms of nutritional deficiency to occur in the absence of any other form of malnutrition (Beisel, 1982). For this reason it is a good nutrient to study alone, yet it can sometimes be difficult to rule out the coexistence of infection in the iron deficient (Dallman, 1987; Laxer et al. 1990).

The influence of iron on immune function has generally been considered to be confusing and contradictory. This may be partly due to the difficulty in separating the effects of the nutrient from that of an infection but it is also influenced greatly by the fact that iron functions as an immunostimulant within a specific range and beyond this range in either direction iron exerts definite immunosuppressive effects. In vitro work by Keown and Descamps-Latscha (Keown and Descamps-Latscha, 1983), Bryan and Leech (Bryan and Leech, 1983), and Matzner (Matzner et al. 1979) illustrating the immunosuppressive potential of various iron salts and iron-containing compounds probably can be explained by the high levels of the mineral in the test environment while numerous studies also exist which report various aspects of depressed immune response associated with iron deficiency (Dallman, 1987; Beisel, 1982).

Iron deficiency itself has been shown to be associated with a depressed immune response but also an unaltered immune response depending upon the study in question. In iron deficient states immunoglobulin levels have been assessed by some and found to be normal (Dallman, 1987; Prema et al. 1982; Bagchi, Mohanram and Reddy, 1980; Sawitsky, Kanter and Sawitsky, 1976; Chandra, 1975a; Chandra and Saraya, 1975; Macdougall et al. 1975) as have some aspects of the cell mediated immune response. It

has been claimed that there is no drop in the proliferative response of lymphocytes on exposure to mitogens in the iron deficient (Gupta, Dhatt and Singh, 1982; Suskind et al. 1977; Gross et al. 1975; Kulapongs et al. 1974; Chandra, 1973) and that phagocytic cells retain their ability to ingest and kill various bacteria (Suskind et al. 1977; Macdougall et al. 1975; Kulapongs et al. 1974). These claims do not go uncontested however.

The findings of many studies alternately suggest that iron is extremely important to various aspects of immune function and that in states of iron deficiency the immune response is significantly compromised.

1.6.3.1.2.1 Effects of Deficiency on the Adaptive Immune Response

Some animal work suggests that both lymphocyte number and nuclear structure could change in iron deficient states (Baliga et al. 1981; Fletcher et al. 1975; Jarvis and Jacobs, 1974). A drop in the percentage of total lymphocytes represented by T cells is low in the periphery blood of iron deficient children with the degree of the drop being proportional to the severity of the deficiency (Chandra and Dayton, 1982; Prema et al. 1982; Bagchi, Mohanram and Reddy, 1980; Srikantia et al. 1976; Chandra, 1975a). Some claim to have elevated T cell number in such cases with iron therapy (Bhaskaram, Siva Prasad and Krishnamachari, 1977; Bhaskaram and Reddy, 1975; Heinzerling et al. 1974). Keusch theorizes that the decreased number of T cells could be due to a depressed cellular proliferative response since the enzyme ribonucleotide reductase is iron-containing and it is essential for cellular multiplication (Keusch, 1982). A fairly recent finding that only lymphocytes activated by mitogens (versus nonactivated resting

lymphocytes) bear transferrin receptors and that iron metabolism profoundly changes when these receptors appear also suggests that iron is integrally involved with these processes (Watson et al. 1991; Makinodan, Lubinski and Fong, 1987; Cunningham-Rundles, 1982; Galbraith et al. 1981).

The decreased proliferation of lymphocytes in the iron deficient is a very controversial concept. Some claim that it does not occur yet others have found that it does (Beisel, 1982; Chandra and Dayton, 1982; Sawitsky, Kanter and Sawitsky, 1976; Fletcher et al. 1975; Macdougall et al. 1975; Joynson et al. 1972). It has been suggested that the in vitro testing method often utilized for this assessment could be unreliable in regards to iron (Mainou-Fowler and Brock, 1985). More controlled animal studies in the area could help resolve the issue.

Many studies suggest the loss of DCH in the iron deficient (Kuvibidila, Baliga and Suskind, 1981; Bhaskaram and Reddy, 1975; Chandra, 1975a; Chandra and Saraya, 1975; Macdougall et al. 1975; Joynson et al. 1972). Strauss claims that it is the inflammatory response needed for a positive skin test which is actually depressed by the iron deficiency (Kuvibidila, Baliga and Suskind, 1981).

Also the suggestion of T cell malfunction has been apparent in studies on lymphokine production. There is some evidence to suggest that lymphocytes from iron deficient subjects may support a reduced production of macrophage inhibition factors (Jacobs and Joynson, 1974; Joynson et al. 1972).

The adaptive humoral response may also be affected negatively by an iron deficiency in man. Prema, for example, has found a moderate drop in the percentage of

B cells in anaemic pregnant women (Prema et al. 1982). There appears to be a more dramatic effect on the humoral response of the rat (Kochanowski and Sherman, 1985a; Kochanowski and Sherman, 1985b; Nalder et al. 1972).

1.6.3.1.2.2 Effects of Deficiency on the Innate Immune Response

There is mounting evidence that the phagocytic cells are adversely affected by iron deficiency. A drop in phagocytic function has been noted in iron deficient animals (Mackler et al. 1984; Moore and Humbert, 1984; Chandra, 1975a; Chandra, 1973) and Yetgin claims that it can be reversed with the administration of iron (Yetgin et al. 1979).

Neutrophils from the iron deficient have a reduced capacity to kill ingested bacteria (Chandra and Dayton, 1982; Dionigi, 1982; Chandra and Saraya, 1975). This has been shown to be true for *Staph. aureus* (Yetgin et al. 1979; Chandra, 1975a; Chandra, 1973), *Staph. albus* (Macdougall et al. 1975), and *E. coli* (Walter et al. 1986; Srikantia et al. 1976). Myeloperoxidase enzymes contain iron and they are needed by the phagocytic cells to kill the ingested bacteria and fungi (Beisel, 1982; Keusch, 1982). Hypersegmentation of nuclei has also been noted in neutrophils in the iron deficient suggesting that this deficiency could be interfering with cobalamin or folate metabolism in some way (Beard and Weintraub, 1969).

1.6.3.1.2.3 Iron and Infection

Microorganisms require a supply of iron to survive and proliferate. The body has effective means to restrict the availability of iron to invading microorganisms. During an

infection the body sequesters much of its iron in tissue storage form plus the extracellular body fluids contain proteins with strong binding affinities for iron. These proteins such as serum transferrin can bind iron so that it is not available to the infectious microorganism (Beisel, 1982). Theoretically iron therapy should not increase the incidence of infection or precipitate subclinical infections unless these functions are ineffective such as in states of PCM where levels of iron-binding proteins are low. In such cases or in instances where iron therapy has been aggressive and prolonged enough to essentially saturate both the tissue storage deposits and the extracellular iron-binding proteins, then the level of free iron in circulation could rise and promote growth of the invading microorganism (Weinberg, 1984). Similarly there could be a problem in states of iron overload such as hemochromatosis. Saturated transferrin levels in man are normally less than 35% and so this should not be a common occurrence (Dallman, 1987). There are both animal and human studies which suggest that the administration of an iron supplement decreases morbidity (Lalond and Holbein, 1984; Fuschmann and Ganzoni, 1977; Mackay, 1928). Results of other studies suggest that an increased incidence of infection can accompany iron therapy (Masawe, Mundi and Swai, 1974; Murray et al. 1978; Murray et al. 1975; Kochan, Wasynczuk and McCabe, 1978). To explain this dual phenomenon Beisel suggests that one should look further than the virulence of the pathogen (Beisel, 1982).

Iron not only plays a role in microorganism virulence but is also a necessary factor to support the host's immune response. The iron-related aspects of host defense help to explain many of the clinical and experimental observations. Once supplied with

nutrients an individual can again develop a fever, generate an inflammatory response, and initiate numerous immunological responses which that individual was unable to support in the malnourished state. The reappearance of such defensive mechanisms can explain the apparent emergence of an infection in the newly refed. Therefore although the pathogen might always have been present the malnourished individual can support an immunological response to it only after he/she is fed. The new supply of nutrients by the improved diet though (such as iron) may in addition give a boost to the microorganism(s) in question (Beisel, 1982).

1.6.3.2 Vitamins

Many vitamins have now been shown to affect the immune response in their own right as well as in combination with other nutrients (Beisel, 1982; Nauss and Newberne, 1981). As has been suggested for zinc and iron, the vitamins also probably function positively within a specific range.

Of the fat soluble vitamins, vitamins A and E are generally recognized as having an influence on immune function while there is little evidence to support such a role for vitamins D or K (Beisel, 1982). Many if not all of the water soluble vitamins have been suggested to play some functional role in supporting the immune response. To date the work on thiamin, riboflavin, pantothenate and biotin has been limited (Dionigi, 1982; Gross and Newberne, 1980). Certainly research in the entire area has not been extensive.

1.6.3.2.1 Vitamin A

Although epidemiological data has suggested a relationship between the consumption of vitamin A and its precursors and the decreased incidence of some diseases in certain human populations (Watson et al. 1991; Kok et al. 1987; Menkes et al. 1986), very little conclusive work has been done in the area (Dionigi, 1982) and a cause-and-effect relationship has certainly not been established.

Animals which have experimentally induced deficiency have displayed both normal and depressed antibody responses. Clinical studies have suggested that hypovitaminosis A could be associated with a drop in peripheral T cells, a depressed DCH response, and a reduced capacity of the lymphocytes to proliferate with exposure to mitogens (Gross and Newberne, 1980).

1.6.3.2.2 Vitamin C

Vitamin C has received widespread attention in this area since Pauling's claims of its "super nutrient" functions (Pauling, 1970). Findings are controversial yet do suggest that deficiencies are associated with depressed immune responses (Chandra and Wadhwa, 1989; Dowd et al. 1986; Beisel, 1982; Gross and Newberne, 1980). Vitamin C levels have been positively correlated with natural killer cell activity (Dowd et al. 1986) and may be involved in the synthesis of a thymic humoral factor (Horribin et al. 1979, Dieter, 1971; Dieter, 1969). Much work is yet to be done in order to clarify the functioning of this vitamin in the immune system but suggestions have been made that it may play a role in both humoral and cellular arms of the immune response (Beisel,

1982). Whether megadoses of the vitamin exert immunostimulatory effects, as Pauling suggests, has not been established.

1.6.3.2.3 Pyridoxine

A deficiency of this nutrient has been associated with both a decreased cellular and humoral response (Chandra and Wadliwa, 1989). It probably exerts a stronger effect here than do many nutrients especially in regards to antibody synthesis (Axelrod, 1980; Chandra, 1980b). Axelrod emphasizes the importance of pyridoxine in nucleic acid synthesis and protein biosynthesis and claims that its role in immunity is through this function since the immune system depends so heavily upon an adequate supply of these compounds for the cell division required in response to a foreign stimulus (Axelrod, 1980).

1.6.3.2.4 Folicin and Cobalamin

Metabolically these vitamins function very closely. Many say that the defects seen in a cobalamin deficiency are often actually due to an inavailability of folate coenzymes (Gross and Newberne, 1980; Lakaku et al. 1971; Nixon and Bertino, 1970). The claim has also been made that some of the immunosuppressive effects seen in iron deficiency may be due to the fact that iron deficient states upset folate metabolism (Gross and Newberne, 1976; Gross et al. 1975).

Work with both rats and humans suggests that folate is essential to various aspects of cellular mediated immunity (Gross et al. 1975; Newberne, 1977). Rodent research has

also suggested a role for vitamin B₁₂, independent of folacin, in the development of immunocompetence late in gestation (Newberne, 1977). Folacin and vitamin B₁₂ (either indirectly through folacin or by itself) are necessary for the synthesis of nucleotide precursors and like pyridoxine, are probably needed by the immune response because of its requirement of these components for cell division (Nauss and Newberne, 1981).

1.6.4 Nutrient Excess and the Immune Response

Nutrient excess and nutrient deficiency have both been associated with immunodeficiency (Beisel, 1982; Chandra and Dayton, 1982; Dionigi, 1982; Chandra, 1980a). Nutrient excess applies to both the obese (caloric supply in excess of requirement) and those who are overconsuming individual nutrients with no caloric value.

Chandra has suggested that in excess of one third of obese children, adolescents, and adults display variable impairment of cell mediated immunity and a decrease of the intracellular killing capacity of the PMNL (Chandra, 1981a; Chandra and Kutty, 1980). Gross and Newberne add that obesity is generally accompanied by a decreased resistance to infection (Gross and Newberne, 1980). Chandra notes that often the obese experience a higher level of iron and/or zinc deficiency (Chandra, 1981a; Chandra and Kutty, 1980). If so, this may help to explain the immune dysfunctions experienced.

Myrvik suggests that excess intakes of both saturated and unsaturated fats interferes with the normal functioning of the reticuloendothelial system. Excess intakes of polyunsaturated fatty acids could suppress cell-mediated immune functions. Although

such associations have been noted, definitive studies elucidating the effects of obesity on the immune system are lacking (Myrvik, 1988).

Excesses of various individual nutrients have now been shown to be associated with potential immune dysfunction. Gross and Newberne suggest that elevated levels of both saturated and unsaturated fatty acids can have such detrimental effects on immunity (Gross and Newberne, 1980). Iron excess also could hamper an immune response and elevate one's risk of infection (Dallman, 1987; Beisel, 1982).

Most evidence supporting the immunosuppressive effect of an excess of an individual nutrient relates to zinc. High plasma zinc concentrations have been associated with problems in the functioning of phagocytes (Beisel, 1982; Chandra, 1980a) and high zinc intakes with significant drops in high density lipoprotein cholesterol levels in young men (Hooper et al. 1980). In man high levels of zinc intake may also exert negative effects on copper retention (Bunker et al. 1987; Sandstead et al. 1982) and copper likely plays an essential role in immunity (Beisel, 1982). Work with guinea pigs has also suggested that excess zinc has the potential to inhibit components of the complement cascade (Montgomery, Chvapil and Zukoski, 1979).

1.6.5 The Elderly

Recently the similarities of immune dysfunction which accompany both aging and malnutrition have been noted (Chandra, 1990; Good and Lorenz, 1988; Bogden et al. 1987; Thompson, Robbins and Cooper, 1987; Chandra, 1984; Katz, 1982). The elderly as a group have both a poorer nutritional status and a poorer immunological status than

younger age groups. With a cause-and-effect relationship of malnutrition and immune dysfunction unfolding, it could suggest that some of the loss in immune response seen in the elderly is due to their poor nutritional status.

Although little work has been done to specifically address the malnourished elderly, a number of studies have been conducted which involve the nutritional supplementation of elderly subjects and the monitoring of immune function (Watson et al. 1991; Bogden et al. 1990; Payette, Rola-Pleszczynski and Ghadirian, 1990; Chavance, 1985; Goodwin and Garry, 1982; Duchateau et al. 1981). Grant claims that the only real way to test alterations in immune function with malnutrition is through an intervention trial (Grant, Custer and Thurlow, 1981) and although there may be some problems inherent in this type of research (Beaton, 1983; Exton-Smith, 1982), this is the route generally being followed.

CHAPTER 2

RATIONALE AND OBJECTIVES

2.1 RATIONALE

Proper nutrition has been shown to be a necessity for overall good health and disease prevention. There has been little comprehensive investigation into the nutritional status of the Canadian elderly since the Nutrition Canada Survey of the early 1970's (Health and Welfare Canada, 1973) and with the exception of an extremely small sample of elderly included in the Nutrition Canada Survey, virtually none has been done in Newfoundland in recent years. Considering the growth in numbers of this segment of our population and the fact that this group has the highest incidence of disease, the study of the nutritional state of this group would seem warranted.

Recently, research has suggested that a state of nutritional deficiency could be accompanied by a depressed level of immune functioning. The elderly have been shown to have a high rate of nutritional deficiency and also an immune response which in certain aspects is depressed as compared to immune function studied in younger adults. Therefore could not the depressed nutritional status of the elderly be in some way at least partly responsible for the suboptimal state of immune functioning seen in this group? One way of answering this would be to improve the nutritional status of a group of malnourished elderly and assess their immune system for significant improvements in function.

2.2 OBJECTIVES

The objectives of this study are-

2.2.1 Primary Objectives

1. To assess the nutritional status of an elderly population in metropolitan St. John's.
2. To assess the immunological status of this test group.
3. To attempt to establish a causal relationship between malnutrition and reduced immune functioning in this group.

2.2.2 Secondary Objectives

1. To determine the prevalence of malnutrition in our test group.
2. To qualify the nutritional status of our test group.
 - a) To determine if a difference exists between the prevalence of malnutrition in male versus female subjects.
 - b) To determine if a difference exists between the prevalence of malnutrition in institutionalized versus noninstitutionalized subjects.
 - c) To determine if the prevalence of malnutrition increases with increasing age.
 - d) To assess the dietary consumption of energy, protein, calcium, iron, zinc, and folacin.
3. To improve the nutritional status of our malnourished subjects and monitor changes in specific aspects of the cellular immune response.
 - a) To determine if the percentage of lymphocytes represented by functional T

cells changes significantly.

b) To determine if the percentage of lymphocytes represented by CD4+ cells changes significantly.

c) To determine if the percentage of lymphocytes represented by CD8+ cells changes significantly.

CHAPTER 3

DESIGN OF THE STUDY

3.1 PHASE 1 - NUTRITIONAL AND IMMUNOLOGICAL ASSESSMENTS

205 reasonably healthy elderly adults volunteered to participate in this study. After signing a consent form (Appendix D) each subject initially underwent a nutritional assessment and an immunological assessment. The nutritional assessment was composed of the collection of personal data, anthropometric data, dietary intake data, haematological/biochemical data, physical/clinical data, and morbidity data (Figure 3.1, Materials and Methods, Appendices E-J). The immunological assessment was composed of a Delayed Cutaneous Hypersensitivity Test (DCH) and laboratory analysis of a blood sample (biochemical data) (Materials and Methods, Appendices H and K).

Those subjects deemed to be nutritionally adequate according to the nutritional assessment no longer participated in the study. Those subjects deemed to be malnourished by this battery of tests entered the second phase of the study.

3.2 PHASE 2 - SUPPLEMENTATION PERIOD

Those malnourished subjects agreeing to stay with the study were assigned the appropriate nutritional supplement(s) to deal with the specific nutritional problems revealed by tests of the original assessment (Materials and Methods). The personal physician of a subject was notified before he/she began the nutritional supplement.

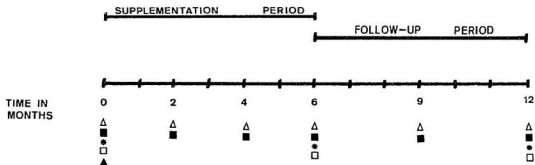
The day on which supplementation began was labelled time 0. The supplement

was taken once daily for a period of six consecutive months (with the exception of vitamin B₁₂) (refer to Materials and Methods). During this period the subject was monitored as is indicated on Figure 3.1. At two month intervals (time 2 months and time 4 months) anthropometric, dietary intake, and morbidity data were collected. At completion of the supplementation period, at time 6 months, all tests were repeated (anthropometric, dietary intake, haematological/biochemical, morbidity, and DCH) as for time 0 with the exception of the personal data.

3.3 PHASE 3 - FOLLOW-UP PERIOD

The supplementation period was followed immediately by six consecutive months of follow-up. During this period no supplement was taken. At time 9 months (three months after the beginning of the follow-up period) dietary intake, anthropometric, and morbidity data were collected. At time 12 months when this period and the progression of the study itself was complete, all tests were performed as at time 6 months (anthropometric data, dietary intake data, haematological/biochemical data, morbidity data, and DCH).

Design of the Experiment



- Δ ANTHROPOMETRIC MEASUREMENTS
- 24 HR. RECALL (3X)
- DCH SKINTEST
- HAEMATOLOGICAL/BIOCHEMICAL TESTS
- ▲ PERSONAL DATA

Figure 3.1

CHAPTER 4

MATERIALS AND METHODS

4.1 SUBJECTS

Two hundred five subjects above the age of 60 years participated in this study. The group included both males and females, both institutionalized and noninstitutionalized. We defined an institution here as an establishment where > 20 persons (not of the same family) are housed and fed as a group. All institutions whose residents participated in this study had at least indirect access to a dietitian.

Participants had either no known medical problem or experienced one that was being successfully controlled to the extent that the condition (hypertension, diabetes, heart disease, or asthma) did not interfere with their normal lifestyle. Those potential subjects presenting chronic bowel disease, chronic chest disease, chronic liver disease, cancer and/or end stage renal disease were ineligible for enrollment.

Subjects all resided in metropolitan St. John's in the province of Newfoundland.

4.1.1 Recruitment of Subjects

After an initial trial of random selection, we proceeded as follows. We approached both community organizations for the aged and nursing homes. In the case of the former, a representative of the community group was approached, an explanatory lecture concerning the study was offered, and after the presentation volunteers were

recruited and asked to sign consent forms before active participation began (Appendix D). In the case of the latter, after approval was obtained from the administration of the institution a social worker provided a short-list of names of residents thought to meet the criteria for recruitment. Each person on that short-list was approached in person and the study was explained in detail. Following this meeting all volunteers signed a consent form.

In addition to the above, we recruited a small number of subjects by physician referral. This resulted from the circulation of an explanatory letter to certain physicians in the area.

Our statisticians agreed to the methods of recruitment used.

4.2 DATA COLLECTION

4.2.1 Personal Data

All volunteers were asked a series of personal questions by an interviewer (Appendix E). The purpose was twofold - 1) to verify their eligibility to participate in the study and 2) to provide data which could help interpret future findings. Questions dealt with such varied issues as birthdate, birthplace, income, formal education, physical exercise, medical/surgical history, domestic environment, and food avoidances.

4.2.2 Nutritional Assessment

The nutritional evaluation of all subjects consisted of anthropometric measurements and analysis of a blood sample. Dietary intake data was also collected. An

early diagnosis of malnutrition was necessary to expedite supplementary feeding. To do this we used anthropometry and haematology/biochemistry as early screening tests. Before notifying each subject of the test results, the intakes of protein, calories, and calcium were estimated.

4.2.2.1 Anthropometry

Anthropometric measurements were used to diagnose PCM in conjunction with certain haematological/biochemical tests (below). The measurements collected height, armspan, skinfold thicknesses at two separate sites (triceps and subscapular), mid upper arm circumference, and total body weight (Appendix G).

Standards used to interpret anthropometric data were specific to the age and sex of each subject (Chumlea, Roche and Mukherjee, 1984).

4.2.2.1.1 Height

Height was measured as the individual stood shoeless against a measure. Height was recorded to the nearest 0.5 centimeter. Three subjects could not stand so their heights were not measured.

4.2.2.1.2 Armspan

Armspan measurements were taken as a second measure of body stature. These were taken with a flexible tape on outstretched arms from the tip of the middle finger on the right hand to the tip of the middle finger on the left hand and recorded to the nearest

centimeter.

4.2.2.1.3 Skinfolts

Exactly half way between the acromion process of the shoulder and the olecranon process of the elbow at the back of the upper arm (left if possible) over the triceps muscle, a small mark was made on the subject's arm. At this point the diameter of a pinch of flesh (double layer of skin with subcutaneous fat between) was measured in triplicate by a skinfold caliper (Lange; Cambridge Scientific Industries, Inc.; Cambridge, Maryland) (Shuran and Nelson, 1986; Grant, Custer and Thurlow, 1981). This triceps skinfold was recorded to the nearest millimeter.

A second skinfold measurement was made of a diagonal pinch of flesh below the scapula on the mid upper back (Shuran and Nelson, 1986; Grant, Custer and Thurlow, 1981). If the position of the scapula was hidden by subcutaneous fat, the subject was directed to bend the elbow of the left arm and swing the bent arm backward so that the lower edge of the scapula would protrude and the measuring site could be located. Subscapular skinfold measurements were made in triplicate to the nearest millimeter.

4.2.2.1.4 Arm Circumference

A flexible tape was used to measure the circumference of the mid upper arm at the mark which had been made over the subject's triceps muscle (refer to section 4.2.2.1.3). The mid upper arm circumference was recorded to the nearest millimeter.

4.2.2.1.5 Body Weight

All individuals were weighed lightly dressed and without shoes on an upright beam balance (Continental Scale Corporation, Bridgeview, Illinois). Weights were recorded to the nearest 0.1 kilogram. Weights were recorded on all but two of the three nonambulatory subjects.

4.2.2.2 Analysis of a Blood Sample

Whole blood was collected from the arm of a sitting subject by venipuncture into three separate sterile vacutainers (Becton Dickinson, Mountain View, California). This procedure was performed by a physician or a certified laboratory technologist. All three blood samples were transported directly to the laboratory for analysis.

One vacutainer was trace element free (Becton Dickinson). This vacutainer was maintained in an upright position when it contained blood to be sure that no zinc from the stopper at the top of the tube would contaminate the blood sample within. Once in the laboratory this sample was spun in a benchtop centrifuge at 700-900 g for 10-15 minutes at room temperature. The pellet was discarded after the supernatant was withdrawn by a glass acid-washed Pasteur pipette and transferred into a plastic self-capped microcentrifuge tube (Nalge Company, Rochester, New York) in which it was frozen. These serum samples were kept at -20 C (degrees centigrade) for a maximum of six months and then analysed.

A second vacutainer contained whole blood which was allowed to clot. This vacutainer was also spun at 700-900 g for 10-15 minutes at room temperature. The

supernatant was withdrawn by a Pasteur pipette and used as a source of plasma for tests which were to be performed on the noncellular portion of the blood - serum prealbumin, serum albumin, serum ferritin, serum vitamin B₁₂, serum folacin, CRP (C reactive protein), and complement C3.

A third vacutainer contained heparin to prevent the blood from clotting. This sample was used as a source of whole blood for use in determining haemoglobin level, haematocrit, white blood cell count, and tests of immune function.

4.2.2.2.1 Protein/Calories

Serum prealbumin values were derived from automated analyses by a Behring Laser-Nephelometer (Rowe, Anderson and Grab, 1970; Reimer, 1978; Whicher, 1978; Van Es, 1981; Thomas, 1984; Ritzmann and Daniels, 1975; Ritzmann, 1983; and Putnam, 1977 and 1984.). Serum albumin values were derived from automated analyses by a Boehringer Mannheim Hitachi System 705 (Coomas, Watson and Biggs, 1971; Addison and Hales, 1971).

Subjects diagnosed as being protein/calorie malnourished had subnormal values in at least one of the following three categories -

- (a) serum prealbumin < 0.20 g/L
- (b) serum albumin < 35 g/L
- (c) > 2 anthropometric measurements less than or equal to the fifth percentile for their specific age and sex (Chumlea, Roche and Mukherjee, 1984).

4.2.2.2.2 Iron

Serum ferritin was estimated by double antibody radioimmunoassay (Quantimmune Ferritin IRMA, Bio-Rad) (Abraham, 1977; Woodhead, Addison and Hales, 1974; Miles et al. 1974; Jeong, Blakemore, and Lewin, U.S. Patent Number 4,244,940). Haemoglobin and haematocrit were obtained by a Coulter counter.

Positive diagnosis of an iron deficiency for a female was based upon a serum ferritin < 10 mcg/L. A haemoglobin value of < 118 g/L and a haematocrit of < 0.37 were also considered to be abnormal values but alone were not enough to constitute a true positive diagnosis of iron deficiency (Hallberg, 1983). A female subject with a normal serum ferritin value but subnormal haemoglobin and/or haematocrit values was informed of her health status in regards to iron and was advised to take an iron supplement. If the subject responded positively to this then she was considered to be originally deficient in iron.

For a male, a positive diagnosis of iron deficiency was based upon a serum ferritin value of < 23 mcg/L. A haemoglobin value of < 128 g/L and a haematocrit of < 0.42 were considered to be abnormal but as for the female, males with normal serum ferritin values but subnormal haemoglobin and/or haematocrit values were only considered to be deficient in this nutrient if they responded positively to supplemental iron.

4.2.2.2.3 Zinc

Serum zinc was estimated by atomic absorption spectrophotometry (Makino and

Takahara, 1981). The certified reference value of the standard used was 302 mcg/dl (Dode Hospital Supplies) and the mean of the true analyzed values was 307 mcg/dl. A serum value of ≤ 70 mcg/dl was taken to represent zinc deficiency.

Samples were collected periodically throughout the study. Approximately every six months frozen serum samples were analyzed for zinc content.

4.2.2.2.4 Vitamin B₁₂

Serum values for this vitamin and folacin were derived from a dual radioassay of the test serum (Simul TRAC-S Solid Phase Radioassay Kit, Vitamin B₁₂ (⁵⁷Co), Folate (¹²⁵I); Becton Dickinson). A serum vitamin B₁₂ value of < 135 pmol/L was used as a positive sign of deficiency for that specific nutrient.

4.2.2.2.5 Folacin

Serum values for this vitamin were tested simultaneously with vitamin B₁₂ (above). A serum value of < 4.5 mcg/L was used as a positive sign of folacin deficiency.

4.2.2.3 Dietary Intakes

The 24 Hour Dietary Recall (Appendix F) was used to estimate dietary intake. The subject was visited by an interviewer and asked to recall everything eaten or drunk during the previous 24 hours. Assistance was sometimes offered by the interviewer to help the recall process. A chart of two-dimensional drawings representing various serving

sizes was used in the estimation of approximate volumes consumed (Boston Nutrition Associates, Boston, Massachusetts).

At every time in the study protocol where the collection of dietary intake data is indicated, three separate 24 Hour Dietary Recalls would be performed on three days which were as close to each other in time as was possible. A mean for these three values was calculated. Subject visits were usually prearranged for the convenience of study participants. Occasionally three visits could not be arranged with a subject. Under these circumstances as many visits as possible were made.

4.2.3 Immunological Assessment

Functioning of the immune system was assessed by a Delayed Cutaneous Hypersensitivity Test (Alexander and Good, 1977; Fudenberg et al. 1978) and the enumeration of various cell types (known to be of functional importance to the cellular immune response) from a whole blood sample.

4.2.3.1 Delayed Cutaneous Hypersensitivity Test

This test was performed on the inner forearm of all consenting participants where possible.

0.1 ml. of five antigens was administered intradermally after the skin had been swabbed with 70% isopropyl alcohol (Kendall Health Care Products Co., Mansfield, MA). The antigens used for this test were -

- (a) Tetanus toxoid (Connaught Laboratories Ltd.; Willowdale,

Ontario)

- (b) Tuberculin purified protein derivative (Mantoux) (Connaught Laboratories Ltd.)
- (c) Trichophyton (Bencard; Mississauga, Ontario)
- (d) Candida Albicans (Bencard)
- (e) Phytohemagglutinin in injectable saline (90 mcg/1.0 ml.) (Wellcome Diagnostics, Dartford, England).

Forty-eight to seventy-two hours after the antigens had been administered the points of injection were observed. An induration of > 5 mm. in diameter was taken to be positive. Usually the induration, or point of localized swelling, was pink in colour. Pinkness alone did not constitute a positive reaction.

An anergic subject was one with no positive response to any of the antigens administered.

4.2.3.2 Analysis of a Blood Sample

Whole venous blood at room temperature was used as a source of lymphocytes. Purified lymphocytes were tested to establish the percentage of total lymphocytes represented by functional T cells, by CD4+ cells, and by CD8+ cells.

4.2.3.2.1 Purification of Lymphocytes from Whole Blood

The procedure used was a variation of Thompson's (Thompson, 1981).

Whole blood was diluted with 0.9% sodium chloride (NaCl). Carefully 10.0 ml.

of the dilute blood was layered over 4.0 ml. Ficoll-Hypaque (Section 2.3.2.1.1) in a disposable capped 15 ml. centrifuge tube (Becton Dickinson, Mountain View, California). Tubes were then centrifuged at room temperature at 400 x g for 30 minutes. The layer of mononuclear cells was removed with a Pasteur pipette from both centrifuge tubes and pooled in a third clean capped centrifuge tube. These cells were washed 2x in Phosphate Buffered Saline (Section 2.3.2.1.2) at approximately 200 x g for 10 minutes each time. Subsequently the cell suspension was washed one more time in Balanced Salt Solution (Section 2.3.2.1.3) at 200 x g for 10 minutes. All washes were performed at room temperature. Now the cell pellet was resuspended in RPMI 1640 culture medium (Flow Laboratories, McLean, Virginia) containing 10% fetal calf serum (FCS) and could be held in this state at room temperature for up to 16 hours.

When cells were required for testing, the cell pellet was carefully resuspended in the medium. If the cells had been sitting in the medium for more than one hour (approximately) then they were centrifuged at 200 x g for 10 minutes at room temperature and then resuspended in fresh medium.

A 10 mcl. sample of the cell suspension was transferred to a small plastic Snap Cap culture tube (Sigma, St. Louis, MO). To this was added 190 mcl Turk's solution (Section 2.3.2.1.4). The tube was vortexed and a sample of its contents was observed under light microscope (Phase Star - American Optical Corporation, Buffalo, N.Y.) on a hemacytometer (American Optical Corporation) to determine the concentration of cells in the prepared cell suspension. Culture medium was added to or removed from the cell suspension to adjust it to a final concentration of approximately $1-2 \times 10^7$ cells per 1.0

ml. of suspension.

4.2.3.2.1.1 Preparation of Ficoll-Hypaque

To prepare the 9% solution of Ficoll used in this protocol, 9 g Ficoll 400 (Sigma) was weighed out and slowly added to a beaker containing approximately 80 ml double-distilled water. The solution was stirred until the Ficoll was completely dissolved and then made up to 100 ml again with double-distilled water in a volumetric flask.

50% (w/v) Hypaque sodium (Winthrop Laboratories, Aurora, Ontario) was diluted to 34% (w/v) by mixing with the appropriate amount of double-distilled water. 10 parts of the 34% Hypaque was added to 24 parts of 9% Ficoll and the density was adjusted to 1.077 g/cm³.

The Ficoll-Hypaque mixture was filter sterilized (0.22 µm Millipore filter) and kept at 4 degrees C in a bottle covered with aluminum foil.

4.2.3.2.1.2 Preparation of Phosphate Buffered Saline (PBS)

The following salts were weighed out in the amounts indicated below and then dissolved in 400-500 ml double-distilled water. This solution was made up to 1000 ml with double-distilled water in a graduated cylinder.

NaCl	8.00 g
KCl	0.20 g
KH ₂ PO ₄	0.20 g
Na ₂ HPO ₄	0.15 g

4.2.3.2.1.3 Preparation of Balanced Salt Solution (BSS)

BSS was made up just prior to usage from 2 stock solutions held at 4 degrees C and at 10x the concentration that they would ultimately be required. Stock solutions were made up as is indicated below.

Stock #1 (10x BSS)

dextrose	5.00 g.
KH_2PO_4	0.30 g.
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	1.79 g.
0.5%(w/v) phenol red solution	10.00 ml.

Dissolve and bring up to 500 ml. with double-distilled water.

Stock #2 (10x BSS)

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.93 g.
KCl	0.30 g.
NaCl	40.00 g.
MgCl_2	0.52 g.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.00 g.

Dissolve and bring up to 500 ml. with double-distilled water.

When BSS was required in the laboratory, 10 ml. of stock #1 and 10 ml. of stock #2 were combined and brought up to 100 ml. with double-distilled water. The 1x BSS was occasionally checked to assure that the pH was 7.2-7.4.

4.2.3.2.1.4 Preparation of Turk's Solution

The following ingredients, in the amounts indicated, were dissolved in 98.5 ml. distilled water.

gentian violet	0.1 g.
95% ethanol	1.0 ml.
acetic acid	0.5 ml.

4.2.3.2.2 T Lymphocyte Count

T cells, as a percentage of total lymphocytes, were derived from a microscopic observation of purified lymphocytes exposed to sheep red blood cells (sRBC) (Thompson, 1981).

100 mcl. of the T lymphocyte suspension (Section 4.2.3.2.1) was added to 100 mcl. of AET (2-aminoethylisothiuronium bromide) treated sRBC (Section 4.2.3.2.1). This was then incubated at 37 degrees C for a period of 15 minutes. During the incubation period the cell suspension was gently rotated.

Next the mixture was centrifuged at room temperature for 10 minutes at approximately 200 x g. The resultant pellet was held at 4 degrees C for 15 minutes and then gently resuspended on a rotator. 4 mcl toluidine blue (0.04-0.06% in normal saline) was added and as soon as the dye had distributed itself throughout the cell suspension, a drop of the suspension was transferred to a microscope slide and observed under a light microscope.

Lymphocytes retained the stain. Red blood cells remained unstained. The sRBC

formed rosettes immediately around the functional T lymphocytes. Here a rosette was taken to be three or more cells adhering to the surface of a stained lymphocyte. At least 200 lymphocytes were counted and the percentage of the total represented by rosetting T cells was calculated.

4.2.3.2.2.1 Preparation of 2-Aminoethylisothiuronium Bromide (AET) - Treated Sheep Erythrocytes

A 3.0 ml. sample of sRBC collected in Alsever's solution (Gibco, Burlington, Ontario) was washed 5 times in normal saline. Each wash was done at 700 x g for 10 minutes at room temperature. The buffy coat was removed and discarded after every wash.

One volume (0.5 ml.) of packed sRBC was added to four volumes (2.0 ml.) AET solution (Section 4.2.3.2.2.1.1). This was mixed and gently rotated at 37 degrees C for 15 minutes. AET treated sRBC were then washed 5x in cold saline. Each centrifugation was carried out at 700 x g for 10 minutes. The cells were made into a 5% suspension in BSS containing 20% FCS. The suspension was held at 4 degrees C for up to 5 days.

4.2.3.2.2.1.1 Preparation of 2-Aminoethylisothiuronium Bromide (AET) Solution

This reagent was made up fresh every time that sheep erythrocytes were to be treated.

A 0.143M solution was used. 0.402 g. AET (Sigma) was dissolved in 8.0 ml. distilled water. The pH of the solution was adjusted to 9.0 using 4N NaOH.

4.2.3.2.3 Enumeration of T Lymphocyte Subsets (CD4+ and CD8+ Cells)

Lymphocytes were prepared as was discussed above (Section 4.2.3.2.1). 10 - 15 mcl. fluorescein isothiocyanate (FITC) conjugated mouse anti-human immunoglobulin (Ig) (Becton Dickinson) was added to 50 mcl. of the lymphocyte suspension. This mixture was held on ice for 15 minutes in the dark. After incubation the cells were washed 3x in cold Dulbecco-phosphate-buffered saline (PBS) (Gibco Laboratories, Grand Island, N.Y.) containing 0.1% (vol/vol) sodium azide. Centrifugation was carried out at 4 degrees C and 400 x g for 10 minutes during each wash. After washing was complete the supernatant was discarded and 1 drop of mounting medium (10% glycerol in PBS) was added to and mixed with the pellet.

A drop of the prepared suspension was transferred to a glass microscope slide and the cells were observed under a fluorescent microscope (Ernst Leitz Wetzlar, West Germany). CD4+ cells stained a different colour than did the CD8+ cells. Lymphocytes which belonged to neither of these subgroups remained unstained. A total of at least 200 apparently viable cells were counted and the percentage of the total represented by CD4+ and CD8+ cells were calculated separately.

4.2.4 Morbidity Data

Morbidity data was collected on all study participants who continued past the assessment phase of the study. The morbidity questionnaire was completed by the interviewer periodically throughout the course of the study (refer to Figure 3.1 and Appendix J). Answers to all questions were filled in by the interviewer in the presence

of the subject. Questions referred to morbidity experienced by the subject during the two weeks immediately prior to the interview only (Martorell et al. 1976).

4.3 DATA HANDLING AND STATISTICAL ANALYSIS

4.3.1 Using Nutritional Status Data to Prescribe Nutritional Supplements

Based upon results of the nutritional assessment (with the exception of the dietary intake data) and interpretation of that data according to the standards used (refer to Materials and Methods below) the subjects were diagnosed as being nutritionally adequate or inadequate. The latter group was supplemented with the nutrient(s) in which each individual was deficient.

Each individual subject was met after the assessment was complete and counselled as to how this deficiency could best be dealt with. Some chose to discontinue with the study and instead planned to follow-up on our findings with their own personal physicians. Those who chose to remain with the study were given the option of following a diet with a more concentrated supply of the deficient nutrient or taking a medicinal supplement on a regular, usually daily basis. Only one of the subjects opted for the altered diet and she dropped out of the study soon after the supplementation period began.

Before receiving any supplement from us the private physician of each subject was notified.

4.3.1.1 Protein/Caloric Malnutrition

PCM was treated with 235 ml. Ensure Plus daily (appendix L) (Ross Laboratories; Montreal, Quebec). Two flavours, chocolate and vanilla, were available. Supplies for each subject were delivered every two months during the study.

Occasionally a subject would not be able to consume the suggested volume of this supplement every day and was then advised to take as much as could be tolerated rather than to stop taking the supplement altogether. Usually at least half of the recommended volume could then be consumed during a 24 hour period.

An attempt at monitoring compliance was made in one of two ways. For nursing home subjects the supplement was dispensed by nursing staff. Subjects living in private homes were asked to keep the empty tins for later collection by the investigator.

4.3.1.2 Iron, Folicin, and Zinc

Iron deficient subjects were given an oral supplement of iron daily in the form of a slow-release tablet (Slow-Fe) (Ciba-Geigy Canada Ltd.; Mississauga, Ontario). Each tablet contained 160 mg. dried ferrous sulfate (equivalent to 50 mg. elemental iron).

If an individual's identified nutritional problem was that of a folicin deficiency then he/she was advised to take a 5.0 mg. tablet of oral folate daily (ICN Canada Ltd.; Toronto, Ontario).

A zinc deficient subject was advised to take one tablet pms-Egozinc (zinc sulphate, equivalent to 50 mg. zinc) (Pharmascience Inc.; Montreal, Quebec) orally every day for the duration of the supplementation period.

For subjects needing supplementation in any of these substances, compliance was monitored in ways similar to the above; i.e. either by an institutionalized procedure in a nursing home or by recheck of empty containers in the case of subjects being followed at home.

4.3.1.3 Vitamin B₁₂

Vitamin B₁₂ was administered to deficient subjects as a monthly intramuscular injection by a registered nurse at a dosage of 100 mcg. (Rubramin) (Squibb Canada Inc.; Montreal, Quebec).

4.3.2 Preparing Dietary Intake Data for Statistical Analysis

Dietary intake data had to be processed for for statistical analysis. Initially recorded food consumptions were manually converted to weights in grams and every different food was assigned a code number which corresponded to the code number assigned to that food in *Nutrient Value of Some Common Foods* (Health and Welfare Canada, 1979). In this form, code number and number of grams in weight, each individual food eaten could be entered into a computer (Equity 11+) (Epson America Inc., Torrance, California) and processed by the program Nutrient Analysis (Behme and Microsystems Research, 1984) to give the amounts of the constituent nutrients consumed. From this program data on the following nutrients was recorded for future analysis - calories, protein, calcium, iron, and folacin. Zinc was also a nutrient of interest but Behme's program did not compute the zinc composition of foods and consequently these

were done by hand (Pennington and Church, 1980).

Once the foods consumed had been broken down into their constituent nutrients, findings for each time period had to be averaged. For example, at time 0 if three separate 24 hour recalls were completed then the average consumption of each nutrient had to be calculated for time 0. Overall values on food consumption refer to average intakes of all time periods during which that subject had participated in the study.

4.3.3 Statistical Analysis

The required number of study subjects was derived by the method of Kraemer and Thiemann in consultation with our statistician (Kraemer and Thiemann, 1987).

Comparing the incidence of malnutrition between subgroups was performed by a Chi-square analysis. Computations were done manually with the use of a 2x2 table.

Comparing changes in immune parameters before and after nutritional supplementation was done by a Student's paired t test. This computation was done with the statistical package MINITAB (Minitab Inc., Pennsylvania, U.S.A.; Schaefer, 1989). A difference with $p < 0.05$ was taken as statistically significant.

All results are reported as mean \pm standard deviation of the mean.

4.4 ETHICAL APPROVAL

This study was approved by the Human Investigations Committee, Memorial University of Newfoundland.

CHAPTER 5

RESULTS

5.1 SUBJECT PARTICIPATION

Two hundred five elderly subjects volunteered to participate in this study. All of these were screened for nutritional status (MATERIALS AND METHODS). Based upon results from the tests used (Table 5.4), 66 were diagnosed as having at least one nutritional deficiency. Ten of these subjects had not expected to find signs of ill health and once informed of their nutritional problem(s) chose to leave the study and seek advice from their own personal physician. By the time we were able to begin the administration of nutritional supplements three other deficient volunteers had developed serious illness, three had died, and three had decided to participate no further for personal reasons. Therefore 47 of the original volunteers entered the supplementation phase of the study (Table 5.1).

Of the 47 participants who began taking a nutritional supplement only 34 successfully completed this phase of the study. Results from the other 13 participants could not be used. Five dropped out for various reasons (Table 5.1) and 8 others showed no improvement in nutritional status by the tests and standards employed.

Three further subjects were unable to complete the follow-up. Two of these subjects died during the course of the follow-up and a third, although she remained with the study for its duration, was unable to complete all of the medical testing required. Therefore 34 subjects entered the follow-up phase of the study and 31 successfully completed it.

TABLE 5.1

Subject Participation

Total # volunteers screened		205
Total # diagnosed as nutritionally deficient		66
# chose personal physician to deal with deficiency	10	
# died	3	
# developed serious illness	3	
# lost interest	<u>3</u>	
Total # dropped out before supplementation period	19	
Total # beginning supplement		47
# did not respond positively to supplement	8	
# developed serious illness	2	
# with perceived side effects to supplement	1	
# lost interest	<u>2</u>	
Total # dropped out during supplementation period	13	
Total # successfully completing supplement		34
# died	2	
# unable to complete appropriate medical testing	<u>1</u>	
Total # dropped out during follow-up	3	
Total # successfully completing follow-up		31

5.2 PERSONAL DATA

Various items of personal data were collected on the subjects (Table 5.2). There were 145 females and 60 males. Of the study subjects, 97 were institutionalized and 108 were living independently. The average age of the group was just under 76 years.

Some of the personal data could not be collected from all of the subjects but yet was obtained from a large number. This data is presented in Table 5.3.

Most subjects were widowed (55%) but approximately one third of those questioned (32.5%) were married. Ten per cent of the subjects had never been married and 2.0% were divorced.

One hundred eighteen of those questioned wore dentures either completely or as a partial plate. Almost 10% still had their own teeth at the time of the study and 5.8% had no teeth but yet chose to wear no dentures.

Most of the subjects (98.4%) had received some degree of formal education (98.4%). The majority of these completed at least a grade 10 or its equivalent (54.0%) while 44.4% did have some formal education but did not achieve a grade 10.

Of the 142 subjects who responded, only 15.0% reported to be regular smokers. Smokers included cigarette smokers, pipe smokers, and tobacco chewers. Forty-four percent reported consuming alcohol but only 2.1% admitted to drinking in excess of two ounces daily.

Physical exercise varied within the group. More than 10% (12.8%) stated that they performed no physical exercise whatsoever while 23.6% claim to partake in physical exercise for more than one hour every day. Exercise for this study group consisted

TABLE 5.2
Personal Data of 205 Study Subjects

Average age - 75.9 years

Age distribution:

60 - 69 years	49
70 - 79 years	87
80 - 89 years	58
90+ years	8

Sex distribution:

Female	145	(average age = 77.0 years)
Male	60	(average age = 73.0 years)

Type of living accommodation:

Independently living	108	(average age = 70.7 years)
Living in an institution	97	(average age = 81.7 years)

TABLE 5.3

Further Personal Data^a

	number	% of total
MARITAL STATUS (n=151)		
widowed	83	55.0
married	49	32.5
single	16	10.6
divorced	3	2.0
DENTAL STATUS (n=139)		
dentures	106	76.3
own teeth	13	9.4
partial denture	12	8.6
no teeth, no dentures	8	5.8
EDUCATION (n=124)		
> grade 11	16	12.9
grades 10 - 11	51	41.1
grades 6 - 9	27	21.8
grades 1 - 5	28	22.6
no formal education	2	1.6

^a This data was not available for all 205 subjects.

TABLE 5.3 (continued)

	number	% of total
SMOKING HABITS (n=142)		
nonsmokers	121	85.0
regular smokers ^b	21	15.0
cigarettes	14	
pipe	5	
chew tobacco	2	
DAILY ALCOHOL CONSUMPTION IN OUNCES (n=141)		
0	79	56.0
< 1	47	33.3
1 - 2	12	8.5
> 2	3	2.1
DURATION OF DAILY EXERCISE IN MINUTES (n=148)		
0	19	12.8
< 15	36	24.3
15 - 29	23	15.5
30 - 44	14	9.5
45 - 59	21	14.2
≥ 60	35	23.6

^b Those smoking < 5 cigarettes per day were not considered to be regular smokers.

TABLE 5.3 (continued)

NUMBER OF DIFFERENT MEDICATIONS CONSUMED PER DAY (n = 87) ^c		
	number	% of total
0	5	5.7
1 - 2	31	35.6
3 - 4	21	24.1
5 - 6	19	21.8
7 - 8	9	10.3
> 8	2	2.3

^c Medications here include laxatives and vitamin supplements apart from those administered as part of this study.

almost entirely of walking and/or light housework but gardening, indoor bicycling, bowling, dancing, and swimming were occasionally reported.

Data on medication consumption was obtained from only 87 subjects. The vast majority of our subjects (94.3%) reported using medications on a regular basis. Although 59.7% were consuming not more than four different medications daily, many consumed more with 2.3% claiming to receive more than eight different medications on a daily basis. Medications here include laxatives and vitamin supplements apart from those administered as part of this study.

5.3 ANTHROPOMETRICS

Body size measurements are presented separately for females (Figure 5.1) and males (Figure 5.2). The subjects in this study were not all of the same age and even within the same sex the average for an anthropometric measurement varies from year to year. Therefore after separating the subjects by sex, the percent of the average for his/her age group was calculated for each individual (Chumlea, Roche, and Mukherjee 1984). Values are presented as mean of these averages \pm one standard deviation of the mean for each measurement taken.

Results in Figure 5.1 show that the mean for every anthropometric measurement taken (weight, height, mid upperarm circumference, triceps skinfold, and subscapular skinfold) was above 100% of the expected mean for females. The means presented in Figure 5.2 for males are all below 100% (with the exception of the weight/height index calculated from the raw data).

Anthropometric Measurements For Females

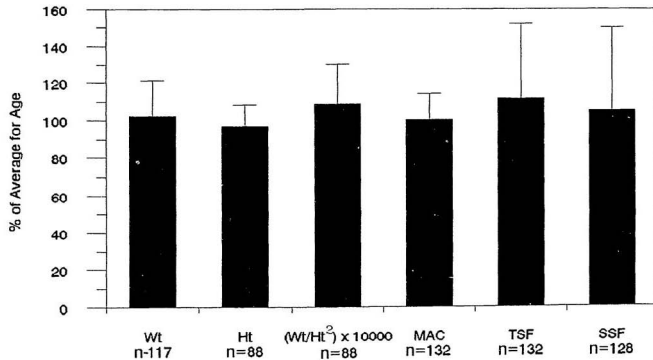


Figure 5.1

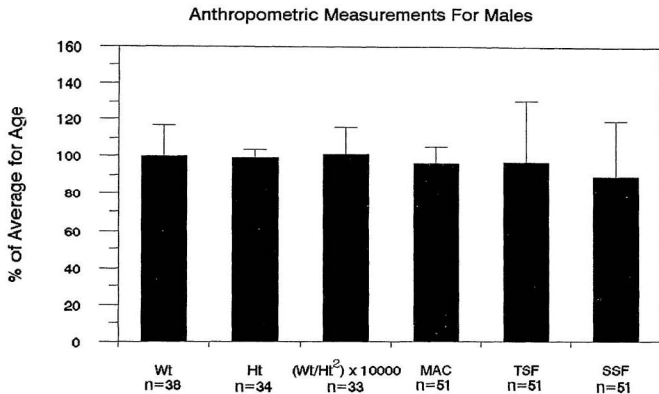


Figure 5.2

The anthropometric data collected on the subjects is variable especially the skinfold measurements. Weight varies considerably from the average for both females and males (refer to standard deviation bars in Figure 5.1 and Figure 5.2). Nineteen of the 117 (16.2%) females weighed in this study exceeded 120% of the average for their age and sex. Six of the 38 (15.8%) males weighed, similarly exceeded 120% of the average for their age and sex.

5.4 NUTRITIONAL DEFICIENCIES

5.4.1 Types of Deficiencies

Clinical signs of nutritional deficiency were rare and so have not been tabulated. Those physical signs which were occasionally noted and which may be related to late stage nutritional deficiencies include such vague indicators as edema (especially in the lower legs and ankles), thin and/or shiny skin, smooth and/or red tongue, pale face and/or conjunctivae, easy bruising, mild lesions at corners of mouth, muscle wasting, and obesity.

Table 5.4 includes the raw data which was used to diagnose deficiencies in the nutritionally deficient. This data is presented in three categories. First data is tabulated for those subjects who were deficient and who successfully completed a nutritional supplement(s). Successfully completing supplementation was a term applied to any subject whose nutritional status in regards to at least one nutrient changed from deficient to normal during the supplementation phase of the study according to the tests and standards used (refer to Materials and Methods).

TABLE 5.4a
Diagnoses of Nutritional Deficiencies in Responders

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard ⁷
MF	iron	Hgb	111	123	-	> 118 g/L
		Hct	0.350	0.362	-	0.37 - 0.47
	folate	serum folate	3.9	> 45	-	> 4.5 nmol/L
EK	iron	Hgb	109	124	129	> 118 g/L
		Hct	0.325	0.36	0.381	0.37 - 0.47
	PCM	serum albumin	34	34	65	35 - 70 g/L
ET	PCM	body weight	47.8	56.0	55.3	55.5 kg
		MAC	237	251	245	252 mm
	iron	serum ferritin	17	57	54	23 - 320 µg/L
		Hgb	123	138	133	> 128 g/L
		Hct	0.361	0.396	0.395	0.42 - 0.52
LK	iron	serum ferritin	7.3	23.0	15.0	10 - 115 µg/L
GC	iron	Hgb	115	120	111	> 118 g/L
		Hct	0.340	0.360	0.334	0.37 - 0.47
EA	vit B ₁₂	serum vit B ₁₂	55	265	310	135 - 710 pmol/L
EB	folacin	serum folacin	4.3	19.6	-	> 4.5 nmol/L

⁷ Standards for anthropometric data are the fifth percentile for the age and sex of the individual.

TABLE 5.4a (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
GC	iron	serum ferritin	17.1	36.0	-	23 - 320 μ g/L
		Hgb	128	145	-	> 128 g/L
		Hct	0.379	0.428	-	0.42 - 0.52
JM	iron	Hgb	118	131	135	> 118g/L
		Hct	0.360	0.392	0.393	0.37 - 0.47
AT	iron	Hgb	128	136	141	> 128 g/L
		Hct	0.380	0.404	0.414	0.42 - 0.52
EG	iron	Hgb	115	120	124	> 118 g/L
		Hct	0.348	0.353	0.365	0.37 - 0.47
CW	zinc	serum zinc	0.55	1.28	0.94	> 0.70 mg/L
GM	zinc	serum zinc	0.61	1.17	0.93	> 0.70 mg/L
		PCM				
		body weight	48.7	52.4	52.3	62.5 kg
		MAC	248	258	244	266 mm
		TSF	5.3	6.2	6.0	13.5 mm
HD	zinc	SSF	7.0	8.0	7.2	10.8 mm
		serum zinc	0.58	1.16	0.86	> 0.70 mg/L
MB	zinc	serum zinc	0.61	1.22	0.754	> 0.70 mg/L
		PCM				
		MAC	245	250	222	250 mm
		SSF	7.0	7.5	7.0	8.0 mm

TABLE 5.4a (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
WH	zinc	serum zinc	0.56	1.52	0.81	> 0.70 mg/LMAF
MAF	zinc	serum zinc	0.43	1.39	1.06	> 0.70 mg/L
	iron	Hgb Hct	117 0.351	136 0.390	130 0.381	> 118 g/L 0.37 - 0.47
AT	zinc	serum zinc	0.58	1.30	1.05	> 0.70 mg/L
JC	zinc	serum zinc	0.65	1.57	1.25	> 0.70 mg/L
IG	zinc	serum zinc	0.64	1.20	1.08	> 0.70 mg/L
PD	zinc	serum zinc	0.70	1.26	0.949	> 0.70 mg/L
AM ⁸	PCM	MAC	240	250	255	243 mm
		SSF	6.7	6.2	8.2	6.8 mm
DM	zinc	serum zinc	0.66	1.69	0.69	> 0.70 mg/L
	PCM	body weight	49.6	50.6	52.9	52.5 kg.
		MAC	230	234	234	258 mm
		TSF	13.6	12.3	11.5	14.2 mm
		SSF	6.3	7.2	7.0	9.0 mm

⁸ Although SSF did not reach the standard by 6 months of supplementation, a borderline body weight was also considered in making this diagnosis of PCM and this value increased by 3.0 kg with supplementation.

TABLE 5.4a (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
EP ⁹	iron	Hgb Hct	117 0.357	130 0.364	129 0.380	> 118 g/l. 0.37 - 0.47
	PCM	MAC	221	222	224	230 mm
TO	zinc	serum zinc	0.69	1.35	1.06	> 0.70 mg/L
WO	zinc	serum zinc	0.62	1.27	0.934	> 0.70 mg/l.
ER	iron	Hgb	110	129	118	> 128 g/l.
		Hct	0.335	0.378	0.352	0.42 - 0.52
		serum ferritin	6.5	23	22	23 - 320 µg/l.
JA	zinc	serum zinc	0.41	0.89	-	> 0.70 mg/l.
EC	zinc	serum zinc	0.56	1.14	-	> 0.70 mg/l.
HC	zinc	serum zinc	0.68	0.99	0.86	> 0.70 mg/l.
	PCM	serum albumin	34	-	36	35 - 70 g/L
		serum prealbumin	0.17	0.15	0.18	0.25 - 0.40 g/l.
WG	folacin	serum folacin	3.6	31.0	> 45	> 4.5 nmol/l.

⁹ Borderline values for TSF, SSF, and serum prealbumin supported the diagnosis of PCM.

TABLE 5.4a (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
AM	zinc	serum zinc	0.61	1.54	0.80	> 0.70 mg/L
IB	iron	Hgb Hct	111 0.336	124 0.370	- -	> 118 g/L 0.37 - 0.47
AS	vit B ₁₂	serum vit B ₁₂	105	305	1140	135 - 710 pmol/L

TABLE 5.4b
Diagnoses of Nutritional Deficiencies in Nonresponders

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
JW	PCM	serum albumin	34	-	30	35 - 70 g/l.
		serum prealbumin	0.20	0.11	-	0.25 - 0.45 g/l.
	folate	serum folate	3.8	3.5	5.3	> 4.5 nmol/l.
EH	folate	serum folate	3.4	4.2	-	> 4.5 nmol/l.
JP	PCM	body weight	43.5	45.1	46.2	47.0 kg
		MAC	212	229	247	247 mm
		TSF	7.2	8.3	10.0	11.7 mm
		SSF	4.5	4.8	4.7	7.3 mm
AL ¹⁰	PCM	TSF	6.0	6.0	-	6.4 mm
MB	PCM	body weight	28.5	32.4	-	48.0 kg
		height	139.5	-	-	151 cm
		MAC	179	187	-	248 mm
		TSF	2.7	2.8	-	12.2 mm
		SSF	3.7	4.0	-	7.7 mm
EB	PCM	body weight	40.6	43.0	43.0	43.0
		MAC	234	223	236	240 mm
		SSF	5.6	4.0	4.7	6.2 mm

¹⁰ This diagnosis was supported by borderline values for SSF and MAC. Body weight could not be assessed accurately for this individual because of a metal leg brace.

TABLE 5.4b (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
MT	PCM	body weight	43.0	43.0	-	49.5 kg
		MAC	230	214	-	252 mm
		TSF	11.3	9.3	-	13.0 mm
		SSF	7.0	6.3	-	8.2 mm
AT	PCM	body weight	45.5	47.4	45.8	49.0 kg
		MAC	237	245	235	251 mm
		TSF	9.3	15.7	13.2	12.7 mm
		SSF	7.5	10.7	6.7	8.0 mm

TABLE 5.4c
Diagnoses of Nutritional Deficiencies in Nonparticipants

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
OR	vit B ₁₂	serum vit B ₁₂	120	-	-	135 - 710 pmol/l.
AP ¹¹	iron	Hgb	115	-	-	> 118 g/l.
		Hct	0.337	-	-	0.37 - 0.47
WS	PCM	body weight	45.0	-	-	48.5 kg
		MAC	182	-	-	237 mm
		TSF	2.7	-	-	5.4 mm
LH	PCM	serum albumin	33	-	-	35 - 70 g/l.
		serum prealbumin	0.25	-	-	0.25 - 0.40 g/l.
UP	iron	serum ferritin	9.6	-	-	23 - 320 µg/l.
		Hct	0.408	-	-	0.42 - 0.52
EJ	PCM	serum albumin	33	-	-	35 - 70 g/l.
		serum prealbumin	0.22	-	-	0.25 - 0.40
MP	iron	serum ferritin	3.8	-	-	10 - 115 µg/l.
	vit B ₁₂	serum vit B ₁₂	90	-	-	135 - 710 pmol/l.
	PCM	body weight	44.8	-	-	46.5 kg
		MAC	217	-	-	246 mm
		TSF	10.0	-	-	11.5 mm

¹¹Borderline ferritin value to support this diagnosis

TABLE 5.4c (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
GM	PCM	body weight	51.3	-	-	55.0 kg
		MAC	235	-	-	250 mm
		TSF	5.0	-	-	6.3
MB	vit B ₁₂	serum vit B ₁₂	110	-	-	135 - 710 pmol/L
MW	PCM	body weight	43.8	-	-	47.0 kg
		MAC	230	-	-	247 mm
		TSF	8.7	-	-	11.7 mm
		serum prealbumin	0.16	-	-	0.25 - 0.40 g/L
	iron	serum ferritin	8.0	-	-	10 - 115 µg/L
		Hct	0.364	-	-	0.37 - 0.47
SH	zinc	serum zinc	0.70	-	-	> 0.70 mg/L
RC	PCM	serum prealbumin	0.19	-	-	0.25 - 0.45 g/L
		MAC	252	-	-	264 mm
		SSF	5.5	-	-	10.0 mm
MS	iron	serum ferritin	8.0	-	-	10 - 115 µg/L
		Hct	0.352	-	-	0.37 - 0.47
	PCM	body weight	43.7	-	-	47.5 kg
		MAC	205	-	-	248 mm
		SSF	4.2	-	-	7.5 mm

TABLE 5.4c (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
JM	zinc	serum zinc	0.68	-	-	> 0.70
WS	PCM	body weight	59.5	-	-	62.5 kg
		TSF	8.0	-	-	8.5 mm
RC	PCM	TSF	7.3	-	-	8.4 mm
		SSF	7.7	-	-	10.7 mm
BH	PCM	body weight	45.5	-	-	47.5 kg
		height	140	-	-	150 cm
		TSF	11.0	-	-	12.0 mm
GM	PCM	height	145	-	-	147 cm
		TSF	9.2	-	-	10.1 mm
		SSF	5.3	-	-	6.5 mm
EP	zinc	serum zinc	0.69	-	-	> 0.70 mg/l.
TC	zinc	serum zinc	0.58	-	-	> 0.70 mg/l.
BH	iron	serum ferritin	7.0	-	-	10 - 115 µg/l.
		Hgb	104	-	-	> 118 g/l.
		Hct	0.329	-	-	0.37 - 0.47
	zinc	serum zinc	0.60	-	-	> 0.70 mg/l.

TABLE 5.4c (continued)

Subject	Deficiency	Test	Value at 0 month	Value at 6 months	Value at 12 months	Standard
EN	zinc	serum zinc	0.66	-	-	> 0.70 mg/L
SR	zinc	serum zinc	0.68	-	-	> 70
AG	PCM	serum albumin	31	-	-	35 - 70 g/L

Table 5.4 presents data separately for the deficient who took the supplement but whose nutritional status still did not meet the criteria for normal after the supplementation period (the nonresponders). Data is also tabulated on the deficient who refused to take the suggested supplement.

For every nutrient studied there were found to be at least five subjects deficient in it (Table 5.5). Protein-calorie malnutrition was the nutritional problem found most often with 27 of the tested subjects found to be affected. Zinc and iron deficiencies were also found in relatively high numbers of the tested elderly, 11.7% and 9.3% respectively. Folic acid and vitamin B₁₂ deficiencies were each found in 2.4% of the population. Confidence intervals of 95% were calculated on each of these percentages. Limits of these confidence intervals are included on Table 5.5.

Of the total number of 205 subjects screened, 32.2% were deemed to be nutritionally deficient by the tests used. Some subjects (6.8%) experienced the deficiency of more than one of the nutrients tested.

5.4.2 Nutritional Deficiency and Sex

There is no significant difference in the prevalence of malnutrition seen in males versus females (Table 5.6). The types of deficiency seen for each sex look similar. With the exception of vitamin B₁₂, deficiencies of all nutrients studied were found in both females and males.

The average age of the female population tested was higher than the age of the male population tested (Table 5.2) and so age adjusted values were calculated and included on Table 5.6. These support the findings of the raw data that no significant difference in the nutritional deficiency profile appears to exist between the sexes.

TABLE 5.5

Types of Malnutrition Observed in 205 Study Subjects

Nutrient	# Deficient	% Deficient (95% confidence intervals)
PCM	27	13.2 (8.5, 17.9)
zinc	24	11.7 (7.2, 16.2)
iron	19	9.3 (5.2, 13.4)
folacin	5	2.4 (0.3, 4.5)
vitamin B ₁₂	5	2.4 (0.3, 4.5)
# subjects with ≥ 1 nutritional deficiency	66	32.2
# subjects with ≥ 2 nutritional deficiencies	14	6.8

TABLE 5.6

Prevalence of Malnutrition with Sex

	FEMALE	MALE
	number (%) (age adjusted %)	number (%) (age adjusted %)
# studied	145 (70.7%) ^a	60 (29.3%) ^a
# deficient	42 (29.0%) (28.3%)	24 (40.0%) (42.9%)
# with ≥ 2 deficiencies	10 (6.9%) (6.3%)	4 (6.7%) (8.6%)
# deficient with PCM	17 (11.7%) (12.4%)	10 (16.7%) (17.1%)
# deficient in zinc	13 (9.0%) (9.0%)	11 (18.3%) (18.8%)
# deficient in iron	14 (9.7%) (8.9%)	5 (8.3%) (10.3%)
# deficient in folacin	3 (2.1%) (1.4%)	2 (3.3%) (5.2%)
# deficient in vitamin B ₁₂	5 (3.4%) (2.7%)	0 (0) --

^aThese values represent % of total. All other percentages represent % within sex.

5.4.3 Nutritional Deficiency and Living Accommodations

When considering the group as a whole, there is no significant difference between the incidence of malnutrition in those residing in institutions versus those residing in private homes (Table 5.7). Although the data could suggest that the incidence of malnutrition is higher in the institutionalized at a rate of 38.1% versus 26.9% in the noninstitutionalized, a chi squared analysis of this data suggests that the difference is not significant. Age adjusted values are also included on Table 5.7 and they support the raw data.

There does appear to be a higher rate of iron deficiency in the institutionalized as compared to the noninstitutionalized - 14.4% versus 4.6% with $0.025 < p < 0.05$ (Table 5.7). This difference is not significant because with the multiple testing involved here, when comparing an individual nutrient such as iron in two different groups then the p value would need to be less than 0.025 to assure that this difference is not due to chance alone. Comparisons between overall groups (institutionalized versus noninstitutionalized, males versus females, etc.) are much stronger.

5.4.4 Nutritional Deficiency with Increasing Age

Excepting zinc, the prevalence of deficiency with regards to one specific nutrient appears either to remain at the same rate with progressing age or to increase (Table 5.8). When considering nutritional deficiency in general its prevalence increases significantly with age (Table 5.8). Figure 5.3 suggests that the increase in malnutrition with age is largely due to its rise after the age of 89 years.

TABLE 5.7

Prevalence of Malnutrition with Different Living Accommodations

	INSTITUTIONALIZED number (%) (age adjusted %)	NONINSTITUTIONALIZED number (%) (age adjusted %)
# studied	97 (47.3%) ^a	108 (52.7%) ^a
# deficient	37 (38.1%) (37.6%)	29 (26.9%) (27.1%)
# with ≥ 2 deficiencies	10 (10.3%) (10.2%)	4 (3.7%) (3.7%)
# deficient with PCM	16 (16.5%) (17.3%)	11 (10.2%) (10.4%)
# deficient in zinc	9 (9.3%) (9.2%)	15 (13.9%) (13.9%)
# deficient in iron	14 (14.4%) ^b (14.2%) ^c	5 (4.6%) ^b (4.7%) ^c
# deficient in folacin	4 (4.1%) (4.1%)	1 (0.9%) (0.9%)
# deficient in vitamin B ₁₂	4 (4.1%) (4.1%)	1 (0.9%) (0.9%)

^aThese values represent % of total. All other percentages represent % within category.

^{b,c}Values with the same superscript are statistically different with $0.025 < p < 0.05$.

TABLE 5.8

Prevalence of Malnutrition with Increasing Age

	60-69 yrs	70-79 yrs	80-89 yrs	90+ yrs
# studied	49(24.3%) ^a	87(43.1%) ^a	58(28.7%) ^a	8(4.0%) ^a
# subjects with ≥ 1 def.	18(36.7%) ^b	24(27.6%) ^b	18(31.0%) ^b	6(75.0%) ^b
# subjects with ≥ 2 def.	4(8.2%)	5(5.7%)	4(6.9%)	1(12.5%)
# deficient with PCM	9(18.4%)	11(12.6%)	5(8.6%)	2(25.0%)
# deficient in zinc	10(20.4%)	7(8.0%)	6(10.3%)	1(12.5%)
# deficient in iron	2(4.1%)	9(10.3%)	6(10.3%)	2(25.0%)
# deficient in folacin	0(0)	2(2.3%)	3(5.2%)	0(0)
# deficient in vitamin B ₁₂	1(2.0%)	1(1.1%)	2(3.4%)	1(12.5%)

^aThese values represent % of total. All other percentages represent % within category.

^bThese values are statistically different when considered as a group with $0.025 < p < 0.05$.

Prevalence Of Malnutrition With Age

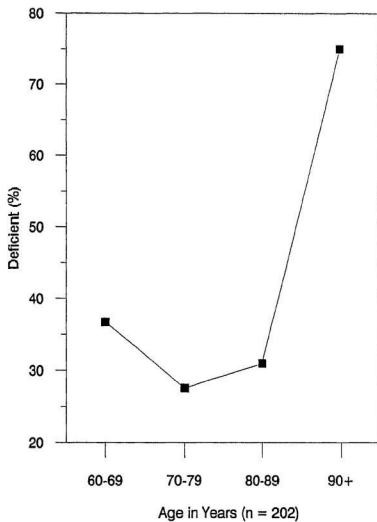


Figure 5.3

5.5 DIETARY INTAKE

5.5.1 Nutrient Intake

A Recommended Nutrient Intake (RNI) is set for each specific nutrient according to age, sex, and physiological status (pregnant, nursing an infant, etc.). For every individual, dietary intake was divided into the intake of specific nutrients and a percentage of the RNI for each nutrient was calculated. Figure 5.4 illustrates the mean plus one standard deviation above the mean for the percentage RNI's consumed for iron, protein, calcium, folacin, calories, and zinc. Mean intakes are above 100% of the recommendations for both iron and protein. Intakes of calcium and folacin are both approximately 85% (85.3% and 84.8% respectively). Caloric intake is only 76.8% of that which is recommended while zinc consumption is the least adequate at 67.3% of the RNI.

Variance in nutrient intake is considerable (Figure 5.4 and Table 5.8) This is especially true for iron, calcium, and protein. Table 5.9 elaborates on the individual variability in the consumption of each of these nutrients.

Table 5.10 describes the relative contributions of carbohydrate, protein, and fat as energy sources in the diets of the subjects. The data is tabulated to present consumption of the institutionalized separately from consumption of those residing in private homes. There is a significant difference in the source of calories between the two groups. The institutionalized consume more calories as carbohydrate and less as fat or protein.

Consumption Of Various Nutrients By Elderly Subjects*

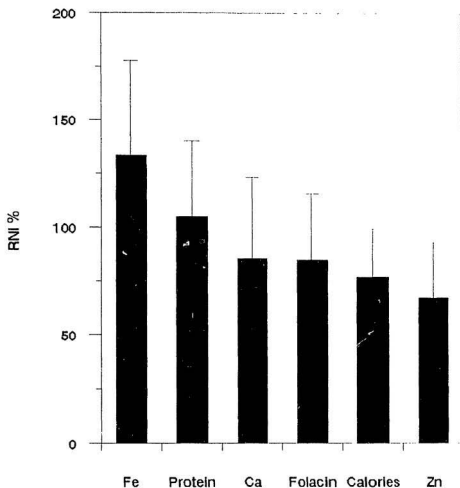


Figure 5.4

* Each bar represents mean and 1 Std. Dev. of the mean, n=140

TABLE 5.9

Variability in Nutrient Consumption

NUTRIENT	# consuming < 66% RNI	# consuming > 100% RNI
iron	4	105
protein	17	66
calcium	49	40
folacin	38	37
energy (calories)	45	20
zinc	79	11

TABLE 5.10

Percentage of Calories Consumed as Carbohydrate, Protein, and Fat

% of Total Calories Consumed as :	Institutionalized (n=60)	Noninstitutionalized (n=49)
Carbohydrate	56.7 \pm 6.79 ^a	53.1 \pm 6.51 ^a
Fat	28.7 \pm 5.51 ^b	31.1 \pm 6.08 ^b
Protein	14.7 \pm 3.14 ^c	15.8 \pm 2.67 ^c

^a significantly different with $p < 0.01$ ($p = 0.0065$)^b significantly different with $p < 0.05$ ($p = 0.033$)^c significantly different with $p < 0.05$ ($p = 0.049$)

Figure 5.5 compares the dietary iron intake of those deemed iron deficient by our tests of nutritional status versus those with adequate body levels of iron. There is no significant difference. Figure 5.6 similarly compares the dietary intake of the zinc deficient versus the zinc adequate. There is no significant difference.

5.5.2 Fibre Intake

Data on fibre consumption was also collected. Figure 5.7 illustrates the mean fibre consumption of zinc deficient subjects versus the mean fibre consumption of those deemed to have adequate body levels of zinc. A students' t test shows no significant difference between the two.

5.6 IMMUNOLOGY

5.6.1 Changes in Immune Function with Progressing Age

In this study various parameters of immune function were measured on elderly subjects but none were collected on younger individuals. Table 5.11 presents percentage of tested subjects with no response to the delayed cutaneous hypersensitivity test and mean values \pm the standard deviation of each mean for complement C3 levels, and percentage of total lymphocytes represented by T cells, CD4+ cells, and CD8+ cells. It also states that over one half (53.8%) of the elderly subjects in this study had detectable circulating levels of the acute phase protein CRP.

Iron Intakes Of Iron Deficient versus Iron Adequate

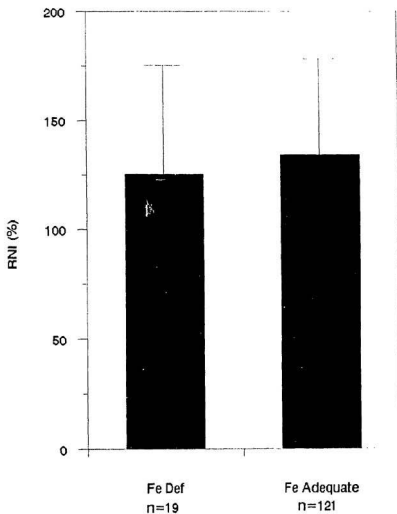


Figure 5.5

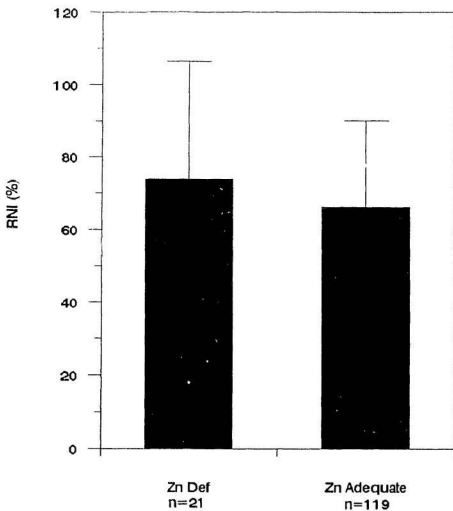
Zinc Intakes Of Zinc Deficient
versus Zinc Adequate

Figure 5.6

Mean Daily Fibre Consumption Of Zinc Deficient* versus Zinc Adequate

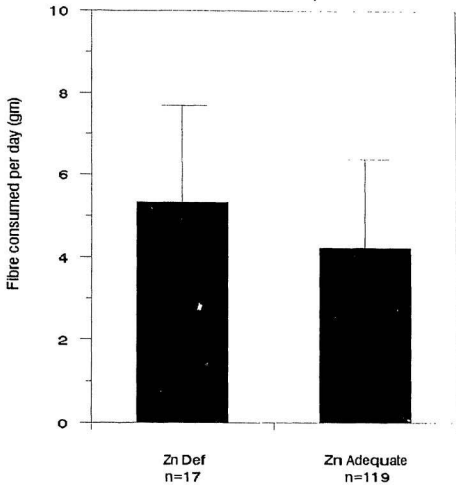


Figure 5.7

* Three of the zinc deficient consumed $< 50\%$ of their RNI for zinc and were excluded here.

TABLE 5.11

Changes in Immune Function with Age

	Data from Present Study on Elderly	Norms from our Laboratory on Younger Subjects	Data from Published Literature on Younger Subjects
T cells	65 ± 10^{ab} (n = 141)	72 ± 7^{ac}	$54 - 70^{ad}$
CD4+ (Leu-3a)	40 ± 12^{ab} (n = 116)	45 ± 10^{ac}	45^{ae}
CD8+ (Leu-2a)	18 ± 8^{ab} (n = 134)	28 ± 8^{ac}	28^{ae}
Complement C3	1.57 ± 0.33^f	-	1.40 ± 0.35^{fg}
% anergic by DCH	36	-	$\approx 5^h$
% with detectable CRP	53.8 (n = 130)	-	-

^a % of total lymphocytes^b mean \pm standard deviation of the mean^c normal range^d Dwyer JM, Bullock WE, Fields JP. Disturbance of the blood t:b lymphocyte ratio in lepromatous leprosy. *New England Journal of Medicine* 1973; 288(20):1036-1039.^e Becton Dickinson Source Book. Becton Dickinson Immunocytometry Systems; Mountain View, California; 1988.^f grams/litre^g Palmblad J, Haak J. Aging does not change blood granulocyte bactericidal capacity and levels of complement factors 3 and 4. *Gerontology* 1978; 24:381-385.^h Wilson JD, Simpson SI. *Diagnostic Immunology and Serology. A Clinician's Guide.* MTP Press Ltd.; Lancaster, England; 1980.

Table 5.11 presents some data collected by other researchers in our laboratory on younger subjects and data published by other groups on some of the same immune parameters measured in young adults. A comparison suggests that our elderly subjects display a poorer response to the delayed cutaneous hypersensitivity skin test than that which is reported for younger aged groups. Percentage lymphocytes represented by CD8+ cells appears somewhat lower than these values in younger subjects while the percentage of total lymphocytes represented by total T cells and CD4+ cells may or may not be down. Complement C3 levels do not appear to be lower in the elderly.

Table 5.11 contains no statistical analysis of data since no raw data was collected on young subjects in this study. This table is merely of descriptive value and allows a superficial comparison of certain aspects of immune function at different ages.

5.6.2 Changes in Immune Function with Nutritional Supplementation

Table 5.12 contains data pertaining only to those subjects who were nutritionally deficient, received six months of nutritional supplementation, and responded positively to that supplementation (Table 5.4). Although there appears to be a trend of increase in all immune parameters tested, the only significant rise with nutritional supplementation is in percentage of total lymphocytes represented by T cells.

Table 5.13 contains data on the members of the group handled by Table 5.12 who were able to complete the subsequent follow-up period. There is no significant difference in any parameter of immune function tested prior to the follow-up period in comparison to after the follow-up.

TABLE 5.12

Changes in Immune Function with Nutritional Supplementation

	Time 0 (Before Supplementation)	Time 6 Months (After Supplementation)
T cells ^a	60.4 ± 12.1 ^b * (n=32)	69.0 ± 11.3 *
CD4+ cells ^a	45.4 ± 15.0 (n=32)	47.1 ± 14.3
CD8+ cells ^a	21.9 ± 8.96 (n=32)	24.2 ± 10.2
Complement C3 ^c	1.51 ± 0.284 (n=22)	1.52 ± 0.296
Total induration of DCH skin test ^d	18.5 ± 10.4 (n=15)	18.6 ± 15.3

* significantly different with $p < 0.01$

^a expressed as % of total lymphocytes

^b all values are presented as mean ± standard deviation of the mean

^c grams / litre

^d millimetres

TABLE 5.13

Changes in Immune Function Following Nutritional Supplementation

	Time 6 Months (After Supplementation)	Time 12 Months (After Follow-up)
T cells ^a	69.7 \pm 11.2 ^b (n=29)	64.7 \pm 10.3
CD4+ cells ^a	48.1 \pm 12.7 (n=30)	51.1 \pm 11.2
CD8+ cells ^a	24.6 \pm 10.7 (n=30)	24.8 \pm 9.47
Complement C3 ^c	1.56 \pm 0.277 (n=24)	1.63 \pm 0.310
Total induration of DCH skin test ^d	17.3 \pm 14.8 (n=12)	17.3 \pm 11.0

^a expressed as % of total lymphocytes^b all values are presented as mean \pm standard deviation of the mean^c grams / litre^d millimetres

For some of the subjects tested a count of total white blood cells was taken. This was not available at time zero but was available for some subjects at time six months and time 12 months. This allowed for the calculation of the absolute number of T cells, CD4+ cells, and CD8+ cells at these times. Table 5.14 portrays some of the data of Table 5.13 but uses absolute numbers of cells in replace of percentages of total lymphocytes. The numbers suggest the same finding that the immune cells monitored did not drop significantly in number during the follow-up.

5.6.3 Changes in Immune Function of the Nonresponders

Eight of the individuals who received a nutritional supplement in this study did not respond positively to the administered supplement (Table 5.4). These were the nonresponders. Although the supplement which they received was not enough to cause a definite improvement in nutritional status according to the tests we employed, they did apparently still exert a small positive influence on the immune response of the nonresponders. Figure 5.8 illustrates the change in the percentage of total lymphocytes represented by T cells with time throughout the study. Only two of the individuals showed no increase in this immune parameter when comparing the value immediately after receiving the supplement (6 months) to zero time. One of the two died shortly after the 6 month testing was performed and this could have had an influence on the results which we obtained. The remaining six responders did appear to be positively affected by the nutritional supplement although this rise in the percent T cells is not a significant rise as was seen in those who did respond better to the nutritional therapy (responders).

TABLE 5.14

Changes in Immune Cell Number Following Nutritional Supplementation

	Time 6 Months (After Supplementation)	Time 12 Months (After Follow-up)
# T cells	4.35 ± 1.56^a (n=25)	3.74 ± 1.44
# CD4+ cells	3.02 ± 1.16 (n=26)	2.94 ± 1.06
# CD8+ cells	1.48 ± 0.825 (n=26)	1.40 ± 0.791

^a all cell counts represent $\# \times 10^9$

Change in % T-Cell of Nonresponders Through Study

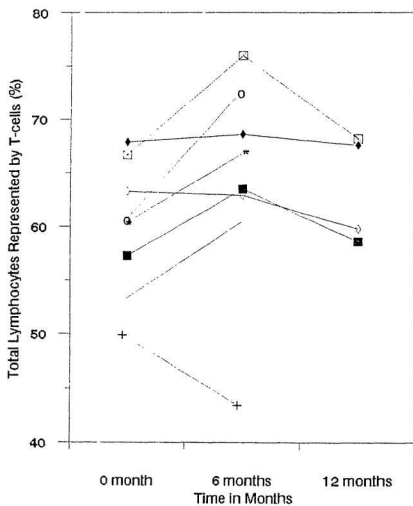


Figure 5.8

CHAPTER 6

DISCUSSION

6.1 HUMAN EXPERIMENTATION

6.1.1 Limitations

Many considerations affect the decision to use human subjects for experimentation. The process can be much more complicated and time consuming than laboratory work with animals or in vitro work yet the applicability of the findings usually makes the extra effort worth while.

In research with humans it is difficult to control for all variables. Even when the subject group is limited to those of a particular age, as in this study, there are many influencing factors which cannot be controlled. The elderly are an extremely heterogeneous group (Chandra, 1990; Masoro, 1990; Schneider et al. 1986; Rivlin, 1982).

There are also ethical constraints in human research. Such restraints will be found in any field but they are much more limiting when humans are studied. For example, duplication of our pre-study values at a three month interval would have been useful but required a delay which on ethical grounds was unacceptable.

In the planning of this study it was obvious that the preferable method of data collection which would involve the simultaneous monitoring of our subjects and a matched control group receiving a placebo in place of the nutritional supplement would not lend itself well to our specific situation for at least two reasons. Firstly, the difficulty

experienced with recruitment limited the total number of participants. The use of subjects of at least 60 years increased the likelihood of legitimate dropout of our subjects from the study due to such circumstances as illness and death. It was anticipated that by putting half of our volunteers in a control group would further limit the number in the experimental group to an unexceptable extent. Secondly it would be very difficult to give a placebo to correspond to the formula which our PCM subjects were to be given.

To compensate for the absence of a control group it was planned that each subject would stand as his/her own control. Three months prior to the collection of the data which is now referred to as time zero data, each individual would be assessed as at time zero. No positive changes in the nutritional and immunological parameters monitored during that initial three month period should help verify any changes observed with nutritional supplementation. Unfortunately our ethics committee refused such a plan, and rightly so, suggesting that once an individual is deemed malnourished that that individual should not have any delay in treatment.

Human subjects must also be given the choice of participation in a study. Unlike animal research or in vitro work this makes human research much more time consuming and it limits the randomness of the subjects regardless of how they are chosen. Those consenting to participate as research subjects tend to be the most interested and educated of those approached (Czajka-Narins et al. 1987; Krause and Mahan, 1984; Exton-Smith, 1982). This makes research of the elderly potentially more difficult since their level of formal education is not as high as it is for many other age groups (Table 5.3) and their knowledge of medical research specifically and its value to mankind is limited. The time-

frame of our study and its requirement for interviewing and testing acted as a deterrent to some of the potential volunteers. A lack of knowledge pertaining to the delayed cutaneous hypersensitivity skin test specifically appeared to influence the choice by many who refused to participate.

Although recruitment for this study was extremely tedious and time consuming one compensation in our elderly subjects was that once committed they were unlikely to drop out. Excluding deaths and the development of serious illness, only two of the subjects who had been given a nutritional supplement did not complete the study (Table 5.1).

6.1.2 Advantages

The numerous variables which influence any human being make it difficult to interpret data collected on human subjects. The control over the experimental situation is limited often by factors which cannot even be identified. Yet even if we as researchers had the potential and ethical approval to control many of the variables which are impinging upon our subjects and thus the outcome of our studies, would it be to our advantage to do so?

Human studies basically involve altering one variable in a subject's lifestyle to see if a change results. It is frustrating work for even if a well-planned study does support a hypothesis the interpretation of this finding remains open to debate, and this debate is complicated by the complexity of human behaviour as well as by ethical constraints upon the researcher.

In laboratory research the situation is controlled and results are therefore generally reliable. Yet human research is one step closer to the truth. It tests a hypothesis in the real life situation. Human research gets close to the question of how the altering of one variable really changes one's lifestyle with all of its composite variables.

6.2 SUBJECTS

In view of the difficulty in obtaining our subjects, it was important to establish and maintain a good rapport with them. Once this trust was established these subjects were quite willing to participate fully in the study. This included the answering of many personal questions.

6.2.1 Personal Data

Our study group was drawn from institutional and noninstitutional sources. Ages ranged from 60 to 96 years, though the number above 89 years was small. Females, as expected in this age bracket, outnumbered males by more than 2:1. Recent statistical approximations suggest that the true ratio of females to males in the Canadian population above the age of 64 years is 1.4 : 1 and is even somewhat lower in Newfoundland (Statistics Canada 1992, Statistics Canada 1986). Those volunteering for participation in this study however had a female : male ratio of 2.4 : 1 (Table 5.2). This makes the finding that the prevalence of nutritional deficiencies is equivalent in males and females (Table 5.6) to be of considerable importance to this study.

6.2.1.1 Marital Status and Level of Formal Education

Although 32.5% of those questioned were still married, the remaining 67.5% were single either by choice or due to death of the spouse (Table 5.3). This is an important factor to note since one of the many variables associated with malnutrition in the elderly is loneliness (Morley, 1986). A low level of education has also been identified in many malnourished elderly and this was also noted in some of our subjects (Table 5.3). Almost 25% of our subjects had achieved no more than a grade 5 education. Both of these factors could contribute to the malnutrition seen in some of our subjects.

6.2.1.2 Consumption of Alcohol and Tobacco

Smoking and alcohol are widely accepted as being detrimental to health. These were probably not major contributors to the health problems of our subjects since only 15.0% regard themselves as regular smokers and approximately 2.0% reported drinking alcohol in excess of two ounces per day (Table 5.3). It has been claimed that heavy drinkers use alcohol in place of nutrient rich foods and this can be of major significance to the elderly who already require a nutrient dense diet to provide adequate levels of all nutrients within a limited caloric requirement. Smokers too may not attend to their diets as the elderly should (Wardlaw and Insel, 1990; Nutrition Reviews, 1990a).

6.2.1.3 Dental Status

It is not just smoking and alcohol consumption which limit nutrient intake in the elderly. Some of the physiological changes which accompany aging may also have a

detrimental effect on food intake. These include such things as loss of taste or loss of teeth (Yeung, Scythes and Zimmerman, 1986; Morley, 1986). Only 9.4% of those studied had their own teeth (Table 5.3). Many of those wearing dentures complained of discomfort in the fit and the inability to chew certain foods. Almost 6% had no dentures or teeth. In summary the dental status of our study group probably is a contributory factor in the prevalence of malnutrition.

6.2.1.4 Physical Exercise

Data was collected on 148 subjects and showed that physical activity was quite variable (Table 5.3). Nineteen (12.8%) of the subjects reported not regularly participating in any type of physical activity. Nine of this group were essentially confined to a wheelchair. For the others it appeared to be simply a matter of choice and the availability of help from attendants. It is interesting to note that 15 of this 19 were female.

The amount of time spent in exercise was more than an hour daily in over 20% of our study group (Table 5.3). This included not only formal exercise but also light housekeeping activities.

We did not analyse this data as it was obtained only by way of gaining an idea of our subjects' lifestyles. We did not attempt to correlate exercise with the obesity seen in approximately 16% of our study group (Section 5.3).

6.2.1.5 Use of Medications

It has been claimed that drugs may alter eating habits and metabolism (Vitale and

Santos, 1985; Tucker et al. 1985; Grant, Custer and Thurlow, 1981; Roe, 1976). Also many drugs have been shown to exert immunosuppressive (Tucker et al. 1985; Solomons, 1979; Grant, Custer and Thurlow, 1981; Sorrell and Forbes, 1975) and immunostimulatory (Gross et al. 1979) effects. In this study both nutritional status and immune function were being followed and therefore it was necessary to monitor drug usage. Any subject using a drug known to interfere with nutritional status or immune function was omitted from the study.

It has been suggested that drug usage and even dependency is common in the elderly (Schneider et al. 1986, Vitale and Santos, 1985; Rivlin, 1982; Roe, 1976). Table 5.3 certainly supports this claim with almost 95% of those questioned reporting the regular use of drugs and a small number reporting the use of more than eight different daily medications.

We know the role that many drugs play in metabolism but undoubtedly there are minor disturbances caused by these drugs which are not widely understood or even realized. Drug utilization is undoubtedly one of those environmental factors which can complicate human research.

6.3 NUTRITIONAL ASSESSMENT

6.3.1 Methodology and Findings

All tests have their inadequacies. To overcome the inadequacies of nutritional testing we decided to have our assessment composed of multiple tests (refer to Materials and Methods). Grant agrees that a good nutritional assessment should be composed of

a number of different tests (Grant, Custer and Thurlow, 1981). Each of the tests tells us something different and together they give us a multifaceted view of nutritional status.

Table 5.4 includes the results of all tests used in the diagnosis of nutritional deficiency in the subjects of this study. These tests were largely anthropometric and biochemical.

6.3.1.1 Anthropometry

Anthropometric measurements are an important part of any evaluation of nutritional health. They give important information concerning protein-calorie malnutrition but they also enable us to monitor overnutrition.

Normal standards are available for many anthropometric measurements taken at various sites around the body. These standards vary with age and sex. The anthropometric standards available specifically for the elderly are limited but all of the anthropometric data which we did collect could be compared to acceptable "elderly specific" standards (Chumlea, Roche and Mukherjee, 1984).

6.3.1.1.1 Height

Height is an indicator of long term nutritional status. Although growth in height would not be severely interrupted by a short term of inadequate dietary intake, a prolonged bout of malnutrition, especially occurring during the growing years, could have a significant negative effect on stature. Our female subjects displayed a mean height of 97.1 % of the average for their age and sex (Figure 5.1). The slight reduction in their

heights in comparison to the expected (100%) could reflect long term nutritional problems yet genetic factors are also important determinants of the stature of a related group (the elderly of metropolitan St. John's). The mean height of the males assessed was determined to be 99.0% of the average for their age and sex (Figure 5.2).

It has been accepted that humans lose height with progressing age. Trotter and Gleser suggest that after physical maturity approximately 1.2 centimetres of height is lost every 20 years (Trotter and Gleser, 1951). This loss of stature is due predominantly to a shortening of the spinal column but the kyphosis experienced by some of the elderly is also a contributing factor (Morley, 1986; Shuran and Nelson, 1986). This loss of stature necessitates the use of height standards specific for the elderly.

In addition to standing height we measured the armspan of our subjects. This is an indicator of stature which should not change with progressing age. The appropriate standards were not available.

6.3.1.1.2 Weight

With progressing age there is a decrease in lean body mass but an increase in adipose tissue. Therefore it is likely to be adipose tissue that accounts for the excess body weight observed in many of our elderly subjects (Morley, 1986; Figure 5.2). The mean weight observed for our elderly females was 102.2%. The mean weight recorded for our male subjects was 99.8% of the average for their age and sex. Neither of these means is considerably different than the expected values of 100% but individual variability was considerable (standard deviations of the means Figures 5.1 and 5.2). Nineteen (16.2%)

of the 117 females weighed and six (15.8%) of the 38 males weighed were obese by the criterion of $> 120\%$ of the expected weight (Stunkard, 1980). Not only can mean values be misleading but so can weight/age which is the value being considered here. Weight/height is often a more reliable indicator of body size. Standards were available on the elderly for an anthropometric index considering both height and weight (Cameron, Roche and Mukherjee, 1984). This index was $\text{weight/height}^2 \times 10000$. The average mean value of our females using this index was 108.7% (Figure 5.1) which better indicates that a number of these women did have excessive body weights. The average mean for males was 100.9% (Figure 5.2).

It is interesting to note that despite association with morbidity under certain conditions, it has been suggested that an excess of body weight in the healthy elderly may have no adverse effect on an individual's rate of survival (Campbell et al. 1990). Burr, Lennings, and Milbank actually claim that an above average body weight may be a favourable prognostic factor in old age (Burr, Lennings and Milbank, 1982).

6.3.1.1.3 Skinfold Thicknesses

Approximately 50% of body fat is subcutaneous (Shuran and Nelson, 1986; Grant, Custer and Thurlow, 1981). Gains or losses in subcutaneous fat probably occur proportionately throughout the body yet the subcutaneous fat layer is known to vary in thickness depending upon age, sex, and fat pad chosen. Therefore to use skinfold measurements as indicators of total body fat it is essential to use good tables of normals for various body sites based on sex and age (Grant, Custer and Thurlow, 1981).

Morley suggests that body fat in males is more accurately determined by skinfold measurements on the trunk (suprailiac and subscapular) yet the more reliable predictors of body fat in females are skinfold measurements on the extremities (biceps and triceps) (Morley, 1986). We were able to obtain good elderly standards on one trunk measurement (subscapular) and one limb measurement (triceps). Yearick showed that both subscapular and triceps skinfolds were positively correlated with relative weight, weight/height, and body mass index (weight/height²) (Yearick, 1978).

Variability in TSF and SSF is obvious for both males and females (Figures 5.1 and 5.2). Both means are less than 100% for males but average 111.6% and 104.8% respectively in females. In accordance with body weight measurements and the weight/height index values, females as a group appear to have more body fat than males. The higher mean value for TSF versus SSF in women suggests that there exists a trend for collecting body fat in the limbs versus the trunk.

6.3.1.2 Biochemical Tests

6.3.1.2.1 Standards

A problem with biochemical testing of the elderly is that a considerable amount of work suggests that the levels of many metabolites and nutrients change with progressing age. Some of these changes may be significant. The difficulty however is that normal standards for the elderly are not yet completely established (Schrijver, Van Veelen and Schreurs, 1985; Exton-Smith, 1982; Mitchell and Lipschitz, 1982a; Mitchell and Lipschitz, 1982b; Dybkaer, Lauritzen and Krakauer, 1981).

6.3.1.2.2 Methodology

The biochemical or laboratory tests for nutritional assessment are relatively quick and easy to perform but are also analytically precise. They are sensitive enough to reveal abnormalities before physical signs of malnutrition are manifested. They are also more accurate than dietary intakes and/or anthropometric measures (Burritt and Anderson, 1984).

Traditionally biochemical tests of nutritional assessment give levels of a given nutrient in a tissue. These are static indices of total body nutriture and are influenced by numerous technical and biological factors. The best of these tissues to analyze would be the storage tissues but these are largely inaccessible and so it is usually a component of the blood which is measured for its nutrient content. Unfortunately the reserves of a nutrient may be depleted before there is a measureable change in the circulating level of that nutrient (Haider and Haider, 1984).

The plasma level of a nutrient sometimes can reflect recent dietary consumption. It would be ideal therefore to collect all blood samples for nutritional assessment from fasted individuals. Unfortunately this is often not possible when large numbers of subjects are involved. The importance of using fasted samples seems to be much more important for individual as compared to group assessment (Harper and Simopoulos, 1982; Sauberlich, 1981). There is some evidence that serum ferritin has a slight diurnal variation (Beaton, Corey, and Steele, 1989) and therefore although we were not able to collect fasted samples in this study whenever possible we made blood collections at approximately the same time of day - late morning.

Both circulating and tissue levels of nutrients are static measures. Researchers have recently suggested that functional tests of nutritional assessment should be carried out to supplement the traditional testing (Haider and Haider, 1984; Chandra, 1981b; Chandra and Scrimshaw, 1980). These tests monitor physiological functions which a nutritional inadequacy should alter. Therefore such a test should indicate not just if the level of a nutrient is not an acceptable average but that the level of the nutrient is not high enough to support a normal biochemical function. Ultimately this is what nutritional assessment is really attempting to achieve - to assess whether a nutrient is present in the body at a level sufficient enough to support the body functions for which it is required. We now know that proper nutrition is essential to support an adequate immune response and many are proposing that some tests of immune function may be good functional tests for nutritional assessment. These include the delayed cutaneous hypersensitivity skin test, complement C3 levels, and T cell enumerations (Chandra and Scrimshaw, 1980). Such functional tests hold much potential but they cannot as yet give information on specific nutrients and so their use should be limited to a general nutritional assessment of groups rather than individuals.

Although a composite of appropriate tests (both static and functional) should enable one to assess nutritional status there are those who suggest that the most reliable indicator of a nutrient's status is to observe the response to a supplement of that nutrient (Solomons, 1979; Grant, Custer and Thurlow, 1981). In this study we have attempted to use all of these methods - static, functional, and intervention.

New and more sensitive tests of nutritional assessment are being developed every

day. This puts researchers of longitudinal studies such as this one at a disadvantage. By the time data is available for analysis a more appropriate method of data collection might be known. It is therefore important for any researcher to be flexible whenever possible but to change a method of data collection well into a study may be neither practical nor advisable. This study took several years to complete. Unfortunately the methods originally chosen for data collection are not necessarily those which would seem to be the most appropriate today.

6.3.1.2.2.1 Iron

Serum ferritin has received a lot of support as a sensitive indicator of iron status (Chandra, 1981b). Indeed Beaton, Corey, and Steele state that it is the preferred test if one is looking at the effects of iron deficiency on function. Serum ferritin is directly related to the level of storage iron in the normal subject (Beaton, Corey and Steele, 1989).

A low serum ferritin was used in this study as a positive indicator of iron deficiency. Loria, Hershko, and Konijn suggest that aging itself could alter ferritin levels (Loria, Hershko and Konijn, 1979). It would therefore be appropriate to use standards of serum ferritin specific to the elderly but as for such values on many body nutrients and metabolites, these standards are not available.

In this study we also measured haemoglobin and haematocrit. Both haemoglobin and haematocrit could drop normally with aging (Thompson, Robbins and Cooper, 1987; Yip, Johnson and Dallman, 1984; Lynch et al. 1982; Lee, Johnson and Lawler, 1981;

Dybkaer, Lauritzen and Krakauer, 1981). We used age specific standards for both of these values yet many studies still refer to younger standards.

Solomons claims that these two values alone are not adequate to diagnose an iron deficiency (Solomons, 1979). If these values really do drop normally with aging and younger standards are utilized then many individuals would be classified as deficient when they are truly normal. In addition the anaemia suggested by low haemoglobin and/or low haematocrit values may be caused by many factors other than a nutritional iron deficiency. Although haemoglobin and haematocrit values are relatively easy to perform and do give important information in conjunction with other tests of iron status such as serum ferritin, alone they are somewhat nonspecific and sensitive only to late stage deficiency (Chandra, 1981). Therefore they cannot be expected to detect as many states of iron deficiency as some other tests such as serum ferritin. Haemoglobin and haematocrit give important background information to support more sensitive tests of iron depletion (Beaton, Corey, and Steele, 1989).

Some of our subjects did display subnormal values of haemoglobin and/or haematocrit without any other sign of nutritional deficiency. Ethically we felt that we should not ignore such subjects for although an iron deficiency could not be definitely diagnosed it could exist in a small number of such patients. Therefore any subject with low values in haematocrit and/or haemoglobin but no other sign of an iron deficiency was offered an oral supplement of iron. A positive response to that supplement was interpreted as an iron deficiency (Table 5.4).

Therefore intervention trials were used in the assessment of iron deficiency as

well as the more utilized static tests of serum ferritin, haemoglobin, and haematocrit. Functional tests were also used in this study of course. Parameters of immune function such as complement C3 level and DCH response were monitored to observe any significant changes with levels of nutritional status. Consequently the value of such immune parameters in portraying nutritional health (nutritional health in general including the contributions of iron, zinc, folacin, and all other nutrients) is indirectly being assessed by this study.

There are many other tests available which can be used in the assessment of iron deficiency. We chose to use those tests (above) which were both adequately informative and practical to our situation but alternate tests could have been performed. For example, Fairbanks and Beutler suggest that in severe iron deficiency that not only is the characteristic microcytic hypochromic anaemia present but that it tends to be accompanied by a drop in the total serum iron level, and a rise in both the free protoporphyrin level of the erythrocytes and the total iron binding capacity (TIBC) of the blood. These latter signs could therefore be assessed as indicators of an established iron deficiency. Unlike serum ferritin concentration though, these latter tests would probably be unable to detect a mild iron deficiency (Fairbanks and Beutler, 1988). Chandra claims that serum ferritin values can drop to less than one third of their normal serum levels with early iron depletion and before any sign of anaemia. Simultaneously there is about a 10% drop in transferrin saturation (a major factor in the TIBC of the blood) and an almost negligible change in free erythrocyte protoporphyrin (Chandra, 1981).

It has been suggested that the major cause of iron deficiency in the elderly is

probably blood loss, especially from the gastrointestinal tract (Herbert, 1990). Therefore stool collection and analysis could have been very informative but not practical for the subjects and study in question.

6.3.1.2.2.2 Zinc

Although there are difficulties with zinc testing (King, 1986), a favoured method for practical reasons is circulating zinc such as plasma or serum zinc (Solomons, 1979). We used the latter.

We also observed the clinical response to supplementation. Of the 21 zinc deficient individuals who agreed to take the supplement, all responded positively to a six month period on medicinal zinc (serum levels increased for all 21). We believe that this response was a confirmation of the original assessment of deficiency (Solomons, 1979).

Prasad has recently claimed success in the development of a more sensitive test for zinc status. He suggests that early stages of deficiency can be detected by monitoring zinc levels in the lymphocytes, granulocytes, and platelets. He also sees some potential in the monitoring of serum thymulin activity (Prasad et al. 1988).

6.3.1.2.2.3 PCM

Both anthropometric and biochemical tests were used in the diagnosis of PCM. The biochemical tests were serum prealbumin and serum albumin. These proteins are widely used as indicators of protein status (Sahyoun et al. 1988; Linn, 1987; Friedman, Campbell and Caradoc-Daires 1985; Mitchell and Lipschitz, 1982b; Fischer, 1981).

Unfortunately the serum concentration of albumin is influenced by a number of factors other than PCM and its long half-life makes it slow to respond to intake yet it is a reliable indicator and an easy test to perform (Mitchell and Lipschitz, 1982a; Grant, Custer and Thurlow, 1981). Serum prealbumin has a shorter half life and so is quicker to indicate a depletion (Fischer, 1981) but the normal values in old age are not really known for either of these serum proteins (Solomons, 1979). Some say that both prealbumin (Kergoat et al. 1987) and albumin levels (Dybkaer, Lauritzen and Krakauer, 1981) drop with progressing age but Mitchell and Lipschitz claim that serum albumin may actually increase with age (Mitchell and Lipschitz, 1982a).

The use of other plasma proteins such as transferrin have also been suggested as indicators of nutritional status. Transferrin has a half life which is intermediate between that of albumin and prealbumin. Its plasma concentration does correlate with short-term changes in protein deficiency but factors other than PCM can affect transferrin synthesis (Fischer, 1981).

6.3.1.2.2.4 Folacin and Vitamin B₁₂

Biochemical estimation of these nutrients is complicated by their close metabolic interrelationship. A test for one may often be influenced by the other (Nauss and Newberne, 1981). A small number of laboratory tests is available for each nutrient (Saubertlich, 1981; Saubertlich, Skala and Dowdy, 1974). We used serum values for each because the methods are both acceptable and practical.

Tissue levels of a nutrient are usually more indicative of body stores than

circulating levels and therefore some chose to monitor red blood cell folate for example, as an indicator of folate status rather than serum folate. Serum levels do drop much earlier than tissue levels but Sauberlich, Skala, and Dowdy suggest that serum folate levels are quite independent of vitamin B₁₂ status yet red blood cell folacin is probably influenced much more by cobalamin (Sauberlich, Skala and Dowdy, 1974).

6.3.1.2.2.5 Calcium

It has been suggested that long term inadequacy of calcium intakes could contribute to osteoporosis. This is of practical importance because of the prevalence of this condition (National Dairy Council, 1982). The assessment of total body calcium might give some insight into the risk of osteoporosis. The problem is that such an assessment of body calcium is very difficult to make.

Calcium is extremely important to many metabolic processes in the body. Levels are kept under rigid hormonal control and therefore do not reflect dietary intake (Hunt and Groff, 1990). Fluctuations in circulating calcium levels would better suggest physiological malfunctions. Consequently there are no adequate biochemical tests for nutritional assessment. Calcium is largely stored in the bone so levels of body calcium can be suggested by tests of bone density but these are often very elaborate and invasive and do not lend themselves well to the routine nutritional assessment of groups (Lutwak, 1981; Sauberlich, 1981; Sauberlich, Skala and Dowdy, 1974).

6.3.1.2.3 Findings

6.3.1.2.3.1 The Overall Prevalence of Malnutrition

Our diagnoses of nutritional deficiencies were made almost entirely by results from biochemical tests. A diagnosis of PCM also required consideration of anthropometric measurements (refer to Materials and Methods).

We were able to find at least a few people deficient in every nutrient we looked at (Table 5.4). The most prevalent nutritional problem observed was PCM. This supports the concept of variability in the elderly for we also noted that a considerable number of those tested were above the average weight for their age, sex, and even height. Approximately 16% were actually obese.

Deficiencies of zinc and iron were also quite prevalent in the group tested at 11.7% and 9.3% respectively. It is difficult to find a cause for these deficient states although multiple factors are probably responsible. The low intakes of zinc likely contributed as did a limited bioavailability from the subjects' diets (Solomons, 1988). Low intakes were probably of less importance for iron but bioavailability could be a factor. Body losses of iron could be partly responsible.

Folacin and cobalamin deficiencies were much less common. It has been suggested that not only folacin and cobalamin are closely related in their metabolic roles but that folacin and iron are similarly related. Some have noted that deficiencies of folacin and iron tend to coexist and this could be the reason (Nauss and Newberne, 1981; Gross and Newberne, 1980). Only five of our subjects showed signs of a folacin deficiency, hardly enough to display a trend. Of this five, one did have a concurrent iron

deficiency and two others had subnormal values of haemoglobin and/or haematocrit.

Based upon the tests used in this study the overall rate of malnutrition seen was 32.2%. Almost one third of all of those tested were shown to be suffering from at least one form of malnutrition. Although the literature suggests that malnutrition is experienced by a relatively large number of elderly this rate is high considering that the elderly assessed here were only those who met criteria which enabled them to be classified as "reasonably healthy". The elderly meeting such criteria would be expected to be those least likely to be malnourished. Our subjects were informed volunteers. Such a subgroup of the elderly tend to be the more inquisitive and better educated - again the type of individual least likely to have nutritional problems.

As often as possible we used standards specific to the elderly. This is a practice not yet always seen in geriatrics. The use of such critical standards would lower the number found to be deficient by more youthful standards.

We monitored only a limited number of nutrients. Considering the number of subjects seen and the voluminous amount of data which already had to be collected to deal with the questions which we hoped to address, it was not practical to handle more. We therefore limited the nutrients monitored to those which we felt were of potentially highest risk in our subject group at the time when this study was originally planned. If we had tested for others we likely would have found more deficiencies. Other researchers have shown the occurrence, in the elderly, of nutritional deficiencies in addition to those addressed here (Morley, 1986; Schrijver, Van Veelen and Schreurs, 1985; Lee, Johnson and Lawlor, 1981).

Therefore there are many reasons to suggest that our estimate of malnutrition in the elderly may be somewhat conservative when it is applied to the elderly as a whole. Due largely to the strict criteria we used for diagnosis, we could have underestimated the number of elderly with malnutrition.

The literature suggests that nutritional deficiencies often occur together (Golden et al. 1978). Our data supports this by finding 14 subjects with multiple deficiencies (Table 5.5).

6.3.1.2.3.2 Malnutrition with Sex, Living Accommodation, and Age

Our data is presented in such a way as to compare nutrients individually and to observe changes in the prevalence of malnutrition with sex, living accommodation, and progressing age. Many publications ponder such comparisons but the raw data is generally not available (Bienia et al. 1982; Rivlin, 1982; Jukes, 1974).

The prevalence of malnutrition did seem to vary considerably from nutrient to nutrient (13.2% for PCM down to 2.4% for folacin and vitamin B₁₂). A chi-squared analysis of the data did not show a significant difference in nutritional status between the sexes nor between the institutionalized and the noninstitutionalized. It did show a significant rise in the prevalence of malnutrition from 60 to 90+ years (Table 5.7). Figure 5.3 illustrates the prevalence with increasing decades of life. It appears that the rise in malnutrition seen with age can be explained by the rise in malnutrition seen specifically in the years after 89. This is of practical significance because it is the 'older elderly' (85+ years) who are increasing most in number (Leveille and Cloutier, 1986).

6.3.1.2.4 Causes of Malnutrition

Why is the prevalence of malnutrition so high in the elderly ? The cause appears to be multifactorial. It generally arises from an impaired intake, uptake, or metabolism of nutrients although an increased requirement of the body for nutrients may also be seen (Yeung et al. 1986; Brown, 1990; Exton-Smith, 1982; Munro, Suter and Russell, 1987; Rosenberg et al. 1982; Czajka-Narins et al. 1987; Morley, 1986; Scrimshaw, Taylor, and Gorden, 1969).

A change in dietary intake can be caused by such factors as lack of health education, decreased physical activity, financial restrictions, and social isolation. Limited education and physical inactivity very likely were contributory factors in our subjects (Table 5.3).

The physiological changes which accompany aging are both numerous and varied (Young, 1990; Munro, 1981; Roland, 1984; Alford and Bogle, 1982). Some of these such as loss of teeth and taste acuity could contribute to malnutrition by limiting food intake. Many of our subjects had lost teeth (Table 5.3). Other physiological changes such as depressed HCl secretion into the stomach and impaired absorption can contribute to problems in nutrient intake (Yeung et al. 1986).

Degenerative diseases and nutrient/nutrient or drug/nutrient interactions may also play a significant role. There is a high rate of degenerative disease in the elderly (Berk and Smith, 1983; Samiy, 1983) and many of these have nutritional side effects. Also drugs can both decrease appetite and affect nutrients after consumption. Our elderly subjects were big drug users (Table 5.3) and many other elderly persons are as well

(Schneider et al. 1986; Rivlin, 1982).

Obviously the cause of malnutrition is multifactorial and complex. We identified a number of probable factors in our subjects but there were probably others present which we did not monitor. The complexity of this problem in the elderly again reflects upon the fact that our study group was very heterogeneous as are the elderly in general.

6.3.1.3 Dietary Intake

6.3.1.3.1 Methodology

Many suggest that dietary intake data is not as precise nor reliable as biochemical data (Krause and Mahan, 1984; Burritt and Anderson, 1984). It is certainly time-consuming to collect and to organise (Black, 1982) yet it provides interesting information which most subjects are willing to give.

Dietary intake data provides information that anthropometric, biochemical, and clinical data cannot because it asks questions of a different aspect of malnutrition (Beaton, 1986; Beaton, 1985; Krause and Mahan, 1984; Appendix M). It is therefore worthwhile in that it provides information which no other form of data collection can provide. Biochemical data provides information on subclinical deficiency. Has a nutritional deficiency progressed to the stage of subnormal circulating levels or interruption of a normal biochemical event? Clinical data addresses late stage malnutrition by asking whether the deficiency has progressed through the subclinical stage to the point where it is now manifesting itself in overt physical abnormalities. Dietary intake data asks about the very early stages of malnutrition. Based upon this intake, what are the

chances that this individual will develop a nutritional inadequacy in the future? Intakes of a nutrient can be quite low without an individual portraying any outward signs of abnormality or even any biochemical disruptions yet the lower these intakes are and/or the longer the time period over which they are consumed at low levels then the higher is the chance that functional impairment will result. Functional impairment should be accompanied by abnormal biochemical and/or clinical signs.

Calcium intake is of obvious relevance to senile osteoporosis. It cannot be monitored by anthropometric or biochemical tests. The collection of dietary intake data is the only practical method of doing this. Therefore dietary intake data was also important in this study to monitor calcium.

Of the various methods available (Beaton et al. 1983; Beaton et al. 1979) we chose to use a 24 hour recall where an interviewer asks about and records all that a subject has consumed in the previous 24 hours. A lot of dietary intake data was collected in this study. It was decided therefore that the 24 hour recall collected by a nutritionist would be the most reliable method. Alternate methods are available but many of these are completed by the individual subject and it was anticipated that the accuracy of such data would drop with the subject's motivation as the study progressed.

All methods of dietary intake data collection have their inadequacies (Beaton and Chery, 1986). The biggest problem with the 24 hour recall method is the day-to-day swings in food intake such that one day's record may not be representative of the normal. In an attempt to overcome this problem, periodically throughout the study (Figure 3.1) we performed a 24 hour recall on three separate days and averaged these. The extra

effort needed for this was a disadvantage yet the extra time spent in conversation with subjects enabled me to develop a positive rapport with them, a major factor in keeping our subjects from dropping out of such a prolonged and oftentimes invasive study. We also collected this data periodically on the same individual over a period of some consecutive months. Tarasuk and Beaton claim that although there may be a sizable random component in the day-to-day consumption patterns of an individual, there appears to be a considerable nonrandom component which becomes apparent on longer term observations of that individual (Tarasuk and Beaton, 1991).

6.3.1.3.2 Findings

Intakes of iron, protein, calcium, folacin, and energy (calories) were obtained through computer analysis. The computer software available to us did not give information regarding zinc or vitamin B₁₂. Zinc status and intakes were of considerable interest to us and so it was computed manually. Vitamin B₁₂ deficiency on the contrary does not affect large numbers at any age group and is usually caused by a physiological abnormality which limits absorption (Carethers, 1988). Dietary intake data for this vitamin would therefore be of minimal importance to us and therefore was not computed.

The three day averages for food intake were divided into intakes per nutrient. For every individual %RNI was then calculated. It is the mean of these %RNI's at time 0 which is illustrated in Figure 5.4.

6.3.1.3.2.1 Protein and Iron

Figure 5.4 suggests that the elderly have an adequate consumption of iron and protein with mean intakes of both being above 100% of the RNI. This in no way suggests that the intakes of all elderly are adequate with regards to these nutrients. Individual variability makes this very unlikely. Indeed the intakes of all nutrients were quite variable. Table 5.8 for example shows that even with a mean consumption of 133.4% of the RNI for iron, four of the individuals tested consumed less than 66% of their respective RNI's. Garry agrees that the intakes of iron and protein are better in the elderly than the consumption of most other nutrients (Garry et al. 1982) while Sahyoun actually found all of her elderly subjects to be consuming greater than 66% of the American RDI (Recommended Dietary Intake) for both of these nutrients (Sahyoun et al. 1988).

In an attempt to see if the dietary intakes of iron really did contribute to the cases of iron deficiency detected in our subject group a comparison was made of the iron intake of the iron deficient versus the iron adequate (Figure 5.5). No significant difference was found between the two nor should any have been expected using such a small number of subjects for this comparison. A trend in higher iron intakes by the iron adequate might be suggested by the data but the large variance in iron intakes and the small numbers concerned mask this possible trend.

6.3.1.3.2.2 Calcium and Folic Acid

The mean intakes of calcium and folic acid were 85.3% and 84.8% of the RNI

respectively. This is not a poor intake overall considering that the RNI is calculated in such a way that it represents the mean intake of a healthy subgroup of the population plus one standard deviation above that mean to allow for individual variability. Therefore if an individual does not consume 100% of the RNI it does not necessarily mean that the intakes are inadequate and will lead to a nutritional deficiency. Yet the further this individual's consumption is below his/her RNI then the higher is the chance that that person will develop a nutritional inadequacy. Although calcium and folacin intakes appear acceptable overall, our concern is with the 49 tested individuals who are regularly consuming less than 66% of their RNI for calcium and the 38 individuals who are similarly consuming such poor levels of folacin (Table 5.8).

Both Garry and Sahyoun found that dietary intakes of calcium and folacin in their elderly subjects were poorer than intakes for iron or protein but in both cases folacin intakes were considerably poorer than calcium intakes (Sahyoun et al. 1988; Garry et al. 1982). For example Sahyoun claims that 52% of her study subjects consume less than 66% of the RDI for folacin but only 4% of her subjects consumed this low a level of calcium. This could be explained by the fact that although Canadian and American dietary recommendations for calcium have been equivalent for some years now the American recommendations for folacin in the early and mid 1980's when Sahyoun and Garry were interpreting their data was much higher than the equivalent Canadian recommendation (400 mcg/day US versus 200 mcg/day Canadian) (Krause and Mahan, 1984). American and Canadian recommendations for folacin intakes are essentially equivalent today (Wardlaw and Insel, 1990).

6.3.1.3.2.3 Calories

The consumption of calories by our subjects was just above 75% of that which is recommended (76.8%) (Figure 5.4). This could be a contributing factor in the cause of PCM which we detected in 27 of our subjects (Table 5.4).

How could such a low energy consumption support the high body weights and the 16% obesity which our anthropometric data showed did exist in this population? Even considering individual variability, Table 5.8 states that only 20 of the 140 individuals interviewed (14.3%) claim to be regularly consuming more than their RNI for energy. Yet 74 of the 155 weighed (47.7%) were above the average weight for their specific age and sex. One possibility is that the low level of physical exercise practiced by many members of this group reduced their calorific requirement to below the average (Table 5.3). A much more likely cause of the discrepancy in data is the underreporting of energy consumption by many of those interviewed.

A big problem associated with dietary intake data is faulty recall. In an attempt to minimize this, the interviewer used a two dimensional picture of food portion sizes. While agreeing that some degree of error is inevitable, Block cites many studies which have shown that intakes reported by 24 hour recalls are more or less accurate for all nutrients with the exception of calories (Block, 1982). Schoeller has also noted this trend in the underreporting of calories consumed and he claims that the worst culprits are the obese (Schoeller, 1990).

6.3.1.3.2.3.1 Nutrients Providing Calories

It is now realized that the source of calories has nutritional significance. Therefore we looked at the energy-yielding nutrients in our subjects' diets to see what proportion of the total calories each one was responsible for contributing. In calculating this data it became obvious that there was a significant difference between the institutionalized and the noninstitutionalized subjects of our study. Therefore Table 5.9 presents this data separately for the two groups.

In the past Canadian intakes of fat have been high with estimates approximating 40% of the calorific value of the diet, with perhaps a slight downward trend in recent trend in recent years. Yet with the growing epidemiological evidence linking high fat diets with various disease states (Dairy Council Digest, 1982; Nutrition Reviews, 1990b; Erickson and Hubbard, 1990) the government of Canada is recommending the further lowering of our fat intakes to 30% of the calories in our diets. Some professionals suggest that a 20% target would be even better but population recommendations must be readily achievable by a large segment of the targeted population in order to be successful. The drop in the consumption of fat should be compensated for by an increased consumption of carbohydrates, especially complex carbohydrates, such that they contribute 55% of the total energy of our diets (Health and Welfare Canada, 1989).

Table 5.9 suggests that although the institutionalized subjects consume a significantly higher portion of their energy as carbohydrate neither group appears to have an inadequately low intake. Even those living in their own homes consume 53.1% of their calories as carbohydrate. Fat consumption is also closer to the recommendations in

the institutionalized as compared to the noninstitutionalized. Both groups are consuming close to the 30% recommended level.

The difference in protein intakes between the two groups is not so great but yet is significantly different ($p = 0.049$). The Canadian government does not make a direct recommendation for protein intake but from the recommendations set for carbohydrate and fat the remaining 15% of the calories in the diet would have to be contributed by protein since it is the only other energy-yielding nutrient. Young suggests that a reasonable protein allowance for the elderly is 12%-14% of total energy intake (Young, 1990). The institutionalized intakes fall closer to this recommendation at a mean of 14.7% yet the intakes of the noninstitutionalized are not a lot higher with a mean intake of 15.8%.

For all three of the energy-yielding nutrients the institutionalized subjects consumed amounts closer to those recommended. This is undoubtedly influenced by the fact that all of the institutions visited either had a dietitian or regular access to one. Although consultation with a dietitian was not able to reduce the incidence of micronutrient deficiencies (Table 5.6) it probably did help in maintaining a good intake of the macronutrients for intakes of the latter are much easier to monitor and control.

Garry similarly assessed the contributions of carbohydrate, fat, and protein to the diets of elderly residing in Albuquerque, New Mexico (Garry et al. 1982). His subjects consumed a comparable amount of protein but more fat (37% of energy versus our 28.7%-31.1%) and less carbohydrate (45%-46% of total energy versus our 53.1%-56.7%) than our subjects. The intakes of our subjects is possibly attributable to secular

trends over recent years in public awareness regarding the health implications of diet.

6.3.1.3.2.4 Zinc

According to Figure 5.4 zinc consumption was the least adequate of all considered with a mean consumption of 67.3% of the RNI. Table 5.8 stipulates that 79 subjects actually consumed less than 66% of the RNI while only 11 consumed more than the recommended amount. Obviously not only is the mean intake of zinc low in the elderly but the low consumption affects a large number.

Both Garry and Sahyoun in their studies on the dietary intakes of the elderly found that vitamin B₆ was the nutrient consumed in the least adequate amount in comparison to recommendations (Garry et al. 1982; Sahyoun et al. 1988). Next to vitamin B₆, consumptions were poorest for zinc and folacin. Obviously poor zinc intakes are not just a problem with our specific study group.

To see how much of an influence dietary zinc intakes had upon zinc deficiency an attempt was made to compare the dietary consumption of zinc by the zinc deficient and the zinc adequate (Figure 5.6). There was no significant difference in the zinc intakes between these two groups. This could support the idea that many factors other than dietary intake contribute to zinc status. The number of subjects used for this comparison is so low that no really significant findings could be anticipated from such a comparison.

King recently has made a proposal which complicates the story of dietary zinc intakes contributing to zinc status (King, 1986). She has suggested that tissue levels of

zinc could affect the body's endogenous losses of the mineral in such a way that if body stores are depleted the body attempts to compensate for this by minimizing losses. If this is indeed the case low intakes need not be expected to precipitate a deficiency.

6.3.1.3.2.5 Probability Analysis

Beaton has attempted to calculate the probability or risk that nutritional deficiency exists in an individual or group based entirely upon the use of dietary intake data (Beaton, 1986; Beaton, 1985). This probability approach is similar to one designed by the US National Academy of Sciences and claims to account for variability due to intake and requirement. There is a considerable variation in dietary intake data due to both an intraindividual and an interindividual component. Requirements can also vary considerably from person to person according to definition (Beaton, 1986; Beaton, 1985). For example, an individual's requirement for vitamin C to prevent clinical signs of scurvy is very different from that same individual's requirement to maintain adequate body stores of vitamin C at a specified level.

The Canadian RNI's have been set based on the assumption that nutrient requirements approximate a normal distribution and with the exception of iodine and iron in menstruating women that the coefficient of variation ($CV = \text{standard deviation} / \text{mean}$, expressed as a percentage) of requirements is approximately 15% of the mean requirement for a specific subgroup. Consequently Anderson, Peterson, and Beaton followed these assumptions of the committee responsible and developed interpretational guidelines for risk assessment (Anderson, Peterson, and Beaton, 1982; Beaton, 1985).

These guidelines allow one to convert the observed level of intake of a nutrient expressed as %RNI to an associated risk of inadequacy.

All males above the age of 60 years share a requirement for zinc and similarly they share a requirement for iron according to the RNI. All females above the age of 60 years also share a requirement for zinc and for iron (Health and Welfare Canada, 1990). By applying our data to Beaton's interpretational guidelines and accepting the assumptions which he has made almost 11 of the 103 females studied are at risk of having inadequate intakes of iron and almost 3 of the 37 males studied could have inadequate intakes of this mineral. Our diagnoses lead to an approximation of 9.3% of those tested to be deficient in iron (Table 5.5). Beaton's approximations via the probability approach are not very different (11/103 and 3/37) considering the number of assumptions inherent in his calculations plus the claims that much of the iron deficiency seen in the elderly may actually be due to causes other than inadequate dietary intake (Herbert, 1991). With the prevalence of inadequate intakes approaching the level of determined deficiency, this could suggest that inadequate intakes are a major factor in the cause of iron deficiency in senior adults.

By applying the method of probability analysis to our collected data on zinc consumption, almost 69 of the 103 females tested and just over 29 of the 37 males tested probably have inadequate intakes of this nutrient. This supports the data illustrated in Figure 5.4 and tabulated in Table 5.9 which suggests that zinc intakes were generally very poor in our study group. It also gives further support to the suggestion that dietary intake was an important contributor to the poor status of zinc seen in a number of our

subjects (Table 5.5).

6.3.1.3.2.6 Possible Interference Contributed by Fibre Consumption

It has been suggested that some minerals consumed are never absorbed into the body because they are bound in the gut by various plant constituents such as phytate. Therefore a high consumption of plant foods may bind up such minerals as iron and/or zinc in the gut and so reduce their absorption. Consumption of such foodstuffs therefore could be a contributor to poor body status of the mineral concerned. Indeed some of the first work done with human zinc deficiency involved the study of young boys in the Middle East in whom an important factor contributing to their zinc deficiency was believed to be their high consumption of bread prepared from high extraction wheat flour and other unrefined cereals (Fraker and Leucke, 1981). These dietary staples contain high amounts of phytate, fibre, lignin, and Maillard products.

The number of our subjects who were specifically deficient in zinc was only 24 (Table 5.4). This may be a high number when considering that only 205 subjects were assessed yet it is a small number to constitute a sample for statistical analysis. Nevertheless, since dietary intake was suggested to be an important possible causative factor we attempted to look further at a factor related to dietary consumption - bioavailability. The difference in fibre consumption of the zinc deficient was compared to its consumption in the zinc adequate by a student's t test (Figure 5.7). It appears that while the difference is not statistically significant the zinc deficient might be consuming somewhat more dietary fibre than the zinc adequate with the former group consuming

a mean of 5.3 grams of dietary fibre per day versus a mean of 4.2 grams for the zinc adequate. Although the computer software available to us could not distinguish the specific sources of fibre in the diet nor the level of accompanying phytate, such factors are important and would be interesting to consider.

The Nutrition Canada Survey found the average daily fibre consumption by males and females above the age of 65 years to be 3.86 grams and 3.30 grams respectively (Health and Welfare Canada, 1973). This intake is comparable with that seen in our subjects but is not a high intake according to more recent estimates. Guthrie suggests that although intake data for fibre is limited current American daily intakes are approximately 6 - 8 grams (Guthrie, 1989).

6.3.1.4 Clinical Data

Attempts were made in this study to record clinical signs of malnutrition from a physical examination of the subjects by a physician. These were by and large nonexistent. Since clinical signs become apparent only when the nutritional deficiency is in a very late stage (Appendix M) this is likely an indicator that the deficiencies found in our subjects were at an early stage, with consequent good prognosis (Krause and Mahan, 1984).

The Nutrition Canada Survey similarly showed that although nutritional deficiencies were detectable in the Canadian population that clinical signs of malnutrition were rarely seen (Health and Welfare Canada, 1973).

Although clinical signs of malnutrition may not lend themselves to early diagnosis of nutritional problems they are important in that they are readily observed by the trained

eye, they can be used to verify findings from other types of testing, and they alone tell us how far the deficient state has progressed. Clinical testing therefore has the potential to contribute to a nutritional assessment (Baker et al. 1982).

6.3.1.5 Morbidity Recall

We also attempted to collect morbidity data on our subjects. Theoretically this data could give our findings real practical significance. In planning this study we had hoped that ultimately we would be able not only to improve the nutritional status and the immune status of our subjects but also to make them feel better as a consequence. This should make it much easier to convince the elderly of the importance of good nutrition yet morbidity data proved very difficult to collect and even more difficult to assess.

Our problems with morbidity stem from its subjective nature. The wellness 'threshold' varies between individuals and even for the same individual at different times. The data as collected could not be relied upon. Martorell has looked at the collection of morbidity data on humans (Martorell et al. 1976) and agrees that the collection of such data has serious limitations. In addition to the problems which we encountered he also claims that people have a tendency to overreport serious illness and underreport minor ailments. Another big problem which he found with this type of data collection was that the underreporting of illness-related events increased as the time lapse between the occurrence and the interview increased. Therefore not only was the data distorted by the severity of the ailment but it was also distorted over time.

6.4 IMMUNOLOGY

6.4.1 Methodology

Basically in this study we wanted to improve the nutritional status of some elderly individuals and monitor their immune function to see if that function did significantly change in some way as a consequence. A decision had to be made regarding what aspects of immune function would be monitored and what tests should be chosen to perform this task. The literature states that although humoral immune function could be changed to some degree in the malnourished by nutritional supplementation the cellular aspects of the immune system hold much more potential (refer to Introduction) to respond positively to such an intervention. Consequently we decided to monitor cellular aspects. At that time the techniques for total T cell, CD4+ cell, and CD8+ cell enumerations had been perfected in our laboratory and C3 levels could be obtained quite readily yet Jensen et al. claimed that "Delayed hypersensitivity skin testing is the single most important means currently available for the clinical evaluation of the status of the cellular immune response ..."¹² Therefore it was decided that this test should also be attempted.

If indeed the improvement of nutritional status does exert a positive influence on cellular aspects of the immune response, then this could be expressed by an enhanced function of immune cells and/or an increase in their number. Many researchers have alternately monitored functional aspects of these cells during times of malnutrition and nutritional repletion. For example, Fraker, Jardieu, and Cook have studied various

¹² Jensen TG, Englert DM, Dudrick SJ, Johnston DA. Delayed hypersensitivity skin testing: Response rates in a surgical population. J Am Diet Assoc 1983; 82:17.

aspects of cellular immune function in nutritionally deprived mice (Fraker, Jardieu, and Cook, 1987). They enumerated splenic lymphocytes but also looked at the intracellular killing capacity of macrophages in vitro and found it to be significantly depressed in the zinc deficient mice. In addition they also examined the responses of B and T cells in vitro to a wide variety of mitogens and the production of interleukin 2 and antibody from these cells respectively. Although they did not detect a significant change in these latter functions as compared to those seen in well fed mice, many researchers have monitored such parameters of cellular immune function in both animals and humans and found various negative influences associated with malnutrition (Chandra and Wadhwa, 1989; Corman, 1985; Chandra, 1983a; Dionigi, 1982) (refer to sections 1.6.1.1.3.2-1.6.1.2).

6.4.1.1 Delayed Cutaneous Hypersensitivity Skin Testing

There is no consensus regarding the selection of antigens for the test but the use of at least five antigens is suggested (Linn, 1987) and these should ideally be chosen with the geographic area of use in mind (Dionigi, 1982). Jensen suggests the use of *Candida*, *trichophyton*, and Tuberculin - Purified Protein Derivative (PPD) as antigens in this test for they have been shown to give good response rates (Jensen et al. 1983). PPD was especially appropriate to our subjects due to the high rate of tuberculosis experienced in this province in the past and the extensive vaccination program which followed. Tetanus toxoid was also chosen as a test antigen since we expected a high previous exposure of our subjects to it. The final antigen administered was phytohaemagglutinin (PHA). PHA is probably not antigenic itself but it causes lymphocytes to undergo blast transformation

intradermally and therefore has the potential to promote a positive DCH response (Gross and Newberne, 1980).

This test of immune function requires the functioning of many immune cells and activities. It is largely a cellular response but not entirely. Because of the complexity of the process and the large number of factors involved a lack of response can be assumed to be likely due to a cellular dysfunction but a detailed interpretation would not be possible (Chandra, 1981b; Cunningham-Rundles, 1982).

6.4.1.1.1 Problems with the Delayed Cutaneous Hypersensitivity Skin Test

Anergy is generally defined as a dermal response of less than 5.0 millimetres in diameter to the antigens administered. It is more common in the elderly than in younger subjects (Lipschitz, 1987; Thompson, Robbins and Cooper, 1987) but it can be caused by many things other than advanced age such as bacterial or viral infections, myocardial infarction, a variety of cancers, and immobilization (Schizgal, 1981). Indeed Grossman suggests that it is acute illness which makes the elderly anergic to a DCH skin test and not age per se (Grossman et al. 1975). He claims that during periods of good health 88% of the elderly will react with an induration greater than 5.0 millimetres to at least one of the test antigens. Even though our subjects met the criteria for 'relatively healthy' 53.8% had detectable levels of CRP (Table 5.11). Since the presence of circulating CRP is usually taken as an indicator of ill health, it is possible that over half of our subjects could be experiencing some degree of illness at the time of testing. Grossman's argument may have some value.

Another difficulty with this test was its intrusiveness, which led to the loss of several potential volunteers. Technical difficulties with the actual administration of antigen was yet another problem, exacerbated somewhat by the change of staff over the long period of the study.

It has been suggested that the repeated administration of the same antigens over time as was necessary in this study could sensitize a subject to these antigens (Shuran and Nelson, 1986; Duchateau et al. 1985). This could also have been an interference to the success of this test in this study but our results were too irregular to establish any trends.

6.4.1.2 Intervention Studies

An intervention study is one of the few methods of experimental research that lends itself well to work on groups of humans. Theoretically it allows the researcher to observe people in their real life setting and to alter only one variable in the situation to see if this alteration has a significant consequence. The variable changed in this study was an increased nutrient(s) consumption due to the supplement administered.

Nutrition intervention studies have been successfully performed on elderly groups (Bogden et al. 1988; Duchateau et al. 1981). Solomons claims that a careful observation of the response to zinc supplementation is the only truly reliable method of assessing zinc deficiency in humans (Solomons, 1979). Its applicability in human nutrition research far surpasses its use in assessing zinc status and recently its values in studying the connection between nutrition and immune response are also being realized. "The immune system is very complex, involving multiple pathways that may or may not be sensitive to nutrition.

Immunocompetence may have nothing to do with malnutrition, and, short of refeeding and retesting, there is no way to determine the relationship clinically."¹³

6.4.2 Senescence of Immune Function with Aging

Table 5.11 includes data which pertains to the level of immune function experienced by the elderly subjects studied here. The means and standard deviations of the means are included for % total lymphocytes represented by total T cells, CD4+ cells, and CD8+ cells. The mean and standard deviation of the mean for recorded complement C3 levels are also included. It is interesting to compare these values with similar values included on the table which apply to younger healthy adults. No statistical analysis could be made since this study did not involve the collection of any data on younger adults yet a comparison of the values tabulated would suggest that the average immune function of our elderly subjects, as suggested by the tests we performed, is less than that which is normally seen in younger individuals. It is important that such a comment be made only of the average immune function of the elderly subjects since the data is obviously quite variable (note standard deviations in Table 5.11) and Chandra has found that the performance of the immune system in some elderly individuals can be just as aggressive as that which is found in some considerably younger adults (Chandra, 1990). He claims that the range in the functioning of the immune system is much broader for the elderly. This is yet another example where a mean value could mask some

¹³Grant JP, Custer PB, Thurlow J. Current Techniques in Nutritional Assessment. Surgical Clinics of North America 1981; 61:460.

important individual findings.

Table 5.11 also states the percentage of our subjects who demonstrated anergy, i.e. with the induration of none of the tested antigens reaching 5.0 millimetres in diameter. The percentage of elderly found to be anergic in this study far exceeds that which one would expect to find in a younger population (Wilson and Simpson, 1980).

Also included in this table is the number of subjects who tested positive for C-reactive protein. It has been argued that malnutrition could lead to a reduced production of CRP yet we found detectable levels in a high percentage of our subjects even though one third of this population tested positive for at least mild malnutrition. Beisel, in support of our findings, has shown that children suffering with PCM are still capable of producing normal levels of CRP (Beisel, Cockerell and Janssen, 1977).

The serum content of CRP can and has often been used as a nonspecific clinical indicator of illness (Dionigi, 1982). It rises in response to such diverse illness as neoplasia, trauma, and infection. The high rate of tested individuals displaying detectable serum CRP therefore could be interpreted as being supportive of the idea that malnutrition and infection and/or illness are indeed very closely interrelated. If this is the case as many third world studies would suggest, then a high rate of infection and/or illness would be expected in this study group with a prevalence of malnutrition being estimated at 33%. We attempted to omit seriously ill subjects from the study but acute illness of varying degrees could be experienced by even the healthiest of individuals and such episodes are unavoidable when individuals are being assessed for prolonged periods of time as in this study. The findings on CRP certainly suggest that this is so.

With a substantial number of subjects having body weights in excess of the expected, one might suggest that this factor could influence the depressed immune responses detected in many of our subjects. There are a number of researchers who have data to support the proposal that obesity could depress immune function (Cunningham-Rundles, 1982; Edelman, 1981; Chandra, 1981a; Chandra and Kutty, 1980).

The purpose of this study was not to address the issue of immune senescence with aging although our findings do appear to give some indirect support for this established phenomenon of aging (Makinodan et al. 1987). It is difficult for even the most well-planned study addressing this issue directly to obtain unequivocal results on the normal aging of the immune system. Fudenberg suggests that this is due to such diverse causes as small sample sizes in many of the studies concerned, different environmental and personal histories of the subjects involved (and being elderly these subjects would have been influenced by these factors for many years), and the virtual lack of longitudinal studies (Fudenberg, 1981).

6.4.3 Response to Nutritional Supplementation

6.4.3.1 Nonresponders

We were unable to raise the nutritional status of all of our subjects from deficient to normal with supplementation (according to the standards set in Materials and Methods). Our aim in this study was to observe changes in immune function with an improved nutritional status. Therefore those who did not respond positively to nutritional therapy could not be monitored for changes in immune function. This group is referred

to as the nonresponders. None of the deficiencies experienced by these individuals was adequately corrected during the supplementation phase.

Details on the eight individuals who composed this group are included in Figure 5.8. All but one of these subjects were diagnosed as being PCM. This is interesting since Mann and coworkers claim that on the basis of their experience with nutrient supplementation of the elderly that there is no reason to expect subjects not to respond unless they are noncompliant (Mann et al. 1987).

We made efforts to assure that compliance was maintained throughout the study by our subjects. Supplements were distributed either directly to the subjects or to a responsible health care worker in the case of some of the institutionalized. This distribution was made on a two month basis with careful instruction to take either one pill and/or one can of supplement daily according to what was required. Further instructions were given to save empty pill bottles and/or cans and return them to the investigator on her next visit. Compliance was good overall but a number of those diagnosed as PCM had difficulty in consuming the full amount of supplement suggested. These subjects were advised to continue taking the supplement in amounts as high as could be tolerated. Very few patients prescribed with PCM had all empty cans at the bimonthly meetings. Therefore noncompliance could have been a contributing factor.

The amount of supplement which the nonresponders received was not enough to normalise their weights but some of them did gain some body size and/or increased serum protein (Table 5.4). Figure 5.8 suggests that although none of this group responded with a significant rise in percent of lymphocytes representing T cells, all but

two showed a trend of increased function of the immune parameter followed with supplementation.

6.4.3.1.1 Level of Supplement

Obviously if the nutritional therapy is not aggressive enough the nutritional status will not improve and therefore one could not expect an improved immune function. Munro suggests that the elderly may actually require a greater amount of supplement to induce the same effect since the uptake of the nutrient by cells may be lower in the elderly (Munro, 1981). Yet overprescription too is a hazard for excessive amounts of some nutrients have been shown to have detrimental side effects such as immunosuppression and increased infection (Chandra and Dayton, 1982; Neumann, 1977; Cunningham-Rundles, 1982). The elderly may be specifically sensitive to high body levels of nutrients for they have a reduced metabolic rate and therefore a prolonged rate of turnover for many nutrients and nutrient metabolites. Excesses of these compounds could therefore pose a potential danger (Nutrition Reviews, 1991; Steffee, 1982).

What is the right dose? This is difficult to answer and varies with the nutrient and the circumstances at hand. For example, Duchateau supplemented her elderly subjects with 220 mg. zinc sulfate twice daily for one month (Duchateau et al. 1981). Bogden found that zinc supplements as low as 100 mg. daily were adequate to raise circulating zinc levels if the administration was maintained for three consecutive months but 15 mg. daily was not sufficient (Bogden et al. 1988). Realizing that both overdose and underdose of supplemental zinc would not be satisfactory we gave the equivalent of 50 mg. zinc for

a period of six months. This dose seemed to be appropriate appropriate with all deficient subjects achieving a normal level of circulating zinc by the end of the six months and no ill effects being noted in any of the subjects.

Judging from response, the levels of all supplements administered in this study, with the possible exception of the caloric dense multivitamin drink for PCM (Ensure Plus - Appendix L), were appropriate. After six months of supplementation only one of those originally identified as being vitamin or mineral deficient still still fitted the criteria for this. The exception was an individual receiving supplemental folacin. In the case of PCM the subjects were unable to consume the volume of the supplement prescribed, so the problem strictly speaking was not in the prescription but in the taking of the supplement.

Not only is the level of supplementation a concern but so is the type of supplementation. This was relatively straightforward in regards to a subject diagnosed to be deficient in a single vitamin or mineral. The subject was administered the individual nutrient as an oral tablet, with the exception of vitamin B₁₂ (refer to Materials and Methods). Even a single nutrient does interact with other nutrients of the body and has the potential to effect the body's metabolism in many ways. A real problem regarding the choice of supplement type was what to give to those subjects diagnosed as PCM. The supplement would have to supply both protein and calories and ideally nothing else. Unfortunately no such supplement is available. We chose Ensure Plus because of its palatability and its content of protein and energy but unfortunately it also contains a certain amount of various other nutrients (appendix L).

6.4.3.2 Responders

6.4.3.2.1 Changes in Immune Response Accompanying Nutritional Supplementation

One aspect of immunity which responded positively to nutrition was an increase in the percent of lymphocytes represented by total T cells. In the group of responders the percentage T cells rose significantly from approximately 60% to 69% during the six months of nutritional supplementation (Table 5.12). With a baseline value of 60 the change of 9 refers to a true rise of 15% ($9 \times 100 / 60$). Even with the small number of subjects under consideration the p value was < 0.01 . The literature claims that other aspects of the cellular immune response which we monitored also have the potential to respond to nutritional therapy (refer to Introduction) but we were unable to detect a significant rise in anything else studied. It could be that these other tested parameters of immune function really do not have the potential to increase significantly with an improved nutritional environment but it could also mean that the additional effect(s) are masked by one or a combination of confounding variables of which numerous always exist when considering human research.

One such variable which must be remembered is that the elderly compose a very heterogeneous group. People age differently. A hypothetical explanation of our findings in light of the limited research data available in this area specific to the elderly is that although certain T cell subsets can respond positively to nutritional supplementation in certain individuals, an increase in total functional T cells is experienced in response to these conditions in a much wider segment of the elderly population.

Belonging to a varied group, elderly individuals display various degrees and types

of malnutrition (Table 5.5). Researchers have introduced the concept that different nutrients may alter immune function in different ways. Possibly the change in immune function experienced by our malnourished group was characteristic of the repertoire of malnutritions experienced by our subjects. Slight differences in immune malfunction seen in other malnourished groups may reflect a slightly different nutritional makeup of their members.

Another factor of importance when attempting to interpret these findings is that malnutrition at different ages has different consequences. For example, young children with PCM maintain immunodeficiencies long after adequate nutritional therapy (Good and Lorenz, 1988) yet in healthy young adults immune capacity seems to be much more reversible with nutritional supplementation (Martin, 1987). Similar responses have been shown for zinc (Fraker, Jardieu and Cook, 1987). Here we have only grouped together those above the age of 60 years but if the elderly constitute as heterogeneous a group as many suggest, then there may be a possibility that the immune functioning of the younger elderly could respond somewhat differently than that of the older elderly. Indeed we have shown that the prevalence of malnutrition varies at different ages within the elderly group (Figure 5.3).

A variable which cannot be easily monitored but is an important consideration here is the effect of the supplement administered to the metabolism of other nutrients. This is of even greater concern in those subjects receiving more than one supplement and especially those receiving Ensure Plus.

Goodwin and Garry raise doubt about the ability of nutritional measures to

improve immune function for prolonged periods of time (Goodwin and Garry, 1983). They claim that successful studies in this area have been of short duration and that the beneficial effect may only be seen transiently. In this study we have shown that the positive effect of nutrition on at least one aspect of immune function can be seen for some months.

6.4.3.2.2 Changes in Immune Response Following Nutritional Supplementation

Table 5.13 suggests that there is no significant difference in the level of any immune parameter measured immediately after supplementation compared to six months later (after the follow-up). This same finding is presented in a slightly different way in Table 5.14 - using the absolute numbers of immune cells rather than their percent of total lymphocytes.

It would probably take more time than six months to detect a significant drop in the percentage of T cells if indeed they have been elevated due to an improved nutritional status. The body has the capacity to store all nutrients to some degree and these stores could maintain a high nutritional status for some time. Until the nutritional status returns to the level which was experienced prior to supplementation, the functioning of the immune system should not be expected to drop to presupplementation levels. By comparing the nutritional indices at 6 months and 12 months in Table 5.4, it appears that the improved nutritional status is generally maintained throughout the follow-up.

Visits with subjects often included discussions of nutritional problems encountered by the individual and how they could be righted. This personal nutrition counselling

might also have had a positive influence on the subjects and could have contributed to the maintenance of good nutritional health in our subjects.

6.4.3.2.3 Complement C3 Levels

There was no significant change in the mean level of complement C3 throughout this study. Although the change in C3 level is not significant it is interesting to note that the recorded values might suggest a slight rise with time through the study, especially from six months to twelve months (Table 5.13). Phair also noted an unexplained rise in C3 levels with time in normal elderly (Phair et al. 1978).

6.4.3.3 Weight Gain and Immune Function

In the elderly weight gain is often an indicator of improved health status. This might apply to our elderly subjects since almost 33% were diagnosed as being PCM. On this premise, we compared immunological data collected on all of those participants who gained weight during the course of the study to those who either maintained their initial body weight or actually lost weight during participation. No significant difference was found between the two groups in either percentage of total T cells, CD4+ cells, or CD8+ cells.

6.5 STUDY DESIGN

The real purpose of research is to gain new knowledge. This study has made contributions not only in its findings relating nutritional status with immune function but

it has also revealed much about methodology. Some such findings were positive and others negative.

This study has shown that in human research it is crucial to know the potential subjects. In this study if it had been anticipated that the seniors approached would be so anxious about participating in the DCH skintest then they would have been approached quite differently. An informational session could have been planned for potential participants prior to recruitment or the study protocol could have been amended to omit this test. Many individuals refused to participate in the study because of this test and it made the process of recruitment difficult. Many problems resulted as a consequence of having a limited number of subjects.

A lesson in methodology which this study also supports is that a researcher should always be flexible. Ideally once our ethics committee rejected our proposal of using subjects as their own controls on the grounds that it would delay treatment to malnourished individuals, we should have developed an alternate plan of collecting control data. A comparison to such data would have made the interpretation of our findings much easier.

A very positive aspect to our protocol was the followup period. The purpose of this was twofold. Data collected after the followup period could give us knowledge concerning the regression of both the immune status and immune function of treated malnourished subjects once treatment was complete. It also allowed us to refer the subject back to his/her physician if indeed a regression was apparent. This allowed the researcher to finish a subject's participation in the study on a positive note.

6.6 PRACTICAL SIGNIFICANCE

The most obvious practical contribution which the findings of this study make are on the well-being of the elderly. We have provided further evidence that good nutrition can have a positive influence on the functioning of the immune system. Better nutritional practice is a small price to pay for an enhanced immune response which could in turn be associated with decreased illness and morbidity in old age. This certainly is of practical value when one considers the high rate of illness experienced by the elderly and also the phenomenal expense of their health care (Schneider, 1983). This is of ever growing importance when one considers the speed at which this subgroup of our society is increasing in number (Grundy, 1983).

Given that improved immune response is beneficial, what is the magnitude of this effect? Will an increase of functional T cells by 15% as we have seen in this study for example, be enough to reduce morbidity in the elderly?

Even small improvements in immune function could be beneficial to the elderly for they are more susceptible to ill health. In the elderly seemingly mild health problems such as influenza can have such serious consequences as death. Therefore even a small boost in immune competence could be of practical significance to many elderly individuals.

Parratt claims that preliminary research findings suggest that in order for the immune response of the average person (regardless of his/her age) to keep abreast of the expanding population of microorganisms to which he/she is exposed that it must function essentially at 100% efficiency (Parratt, 1980). Although it is unlikely that any body

function runs at 100% efficiency, Parratt's claim suggests the importance of maximum efficiency in the immune response. If this is indeed the case, then the contribution which can be made by nutritional factors is conceivably substantial enough to play a practical role.

The association of nutrition and immune response has broader implications than the improvement of well-being in the elderly although that in itself is extremely important. The concept holds much promise for other malnourished groups especially those with possible immune dysfunction.

Natural killer cells are specific cells of the immune system which have been shown to play an important role in a cancer patient's fight against disease (Roitt, Brostoff and Male, 1989). Many cancer patients experience nutritional inadequacies. Righting these nutritional problems could therefore improve their immune response. Indeed recent work has suggested that natural killer cell activities may respond positively to some nutritional factors (Richards and Djeu, 1990).

The outcome of surgery in many surgical patients is often better in those who have received nutritional therapy (Cerra, 1991). The positive influence of nutrients on the functioning of the immune system could help explain this. It is an area with much promise but yet requiring much more work.

Yet another group of individuals who could benefit from the application of such knowledge is those with AIDS. Bogden has shown that a number of patients testing seropositive for HIV-1 have low serum concentrations of a number of nutrients (Bogden et al. 1990). A correction of nutritional problems could boost the functioning of the

immune system in some such individuals.

The efficiency of vaccination programs could improve with this knowledge. If nutrition improves immune response then it should hold true that the better nourished will benefit maximally from vaccination. There is some support for this concept already in both young children (Katz, 1977) and the elderly (Vitale and Santos, 1985).

6.7 SUMMARY AND CONCLUDING REMARKS

Originating with field studies on children in the Third World, there has been evidence suggesting that nutrition and immune function are integrally related. Our study gives further support to this. Specifically we have shown that in malnourished elderly subjects the percentage of total lymphocytes represented by functional T cells can be significantly increased by righting the nutritional deficiency. This finding has practical significance both for the elderly and for other groups who experience malnutrition but yet are attempting to fight off sickness and disease.

The elderly compose an extremely heterogeneous group and although some of them maintain an aggressive immune response many have been shown to house an immune system which loses functional capacity as the years progress. Improving the nutritional health of this group is a practical effort which can improve their immune response and potentially decrease their ill health and the associated discomforts. This is of importance to society as a whole for the elderly compose the segment of our society which experiences most sickness and the cost of their health care is disproportionately large. Also the numbers of elderly are increasing at a faster pace than those of other age

groups. Therefore the knowledge that good nutritional health may serve as a preventive measure against disease and ill health in old age could prove fruitful.

This finding though has implications which surpass the elderly. If this improvement in immune function by nutritional therapy can apply to other malnourished groups, then such therapy could hold potential for many others such as surgical patients, cancer patients, and those suffering from AIDS.

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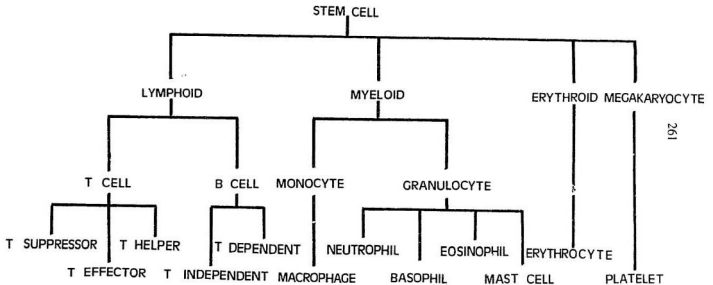
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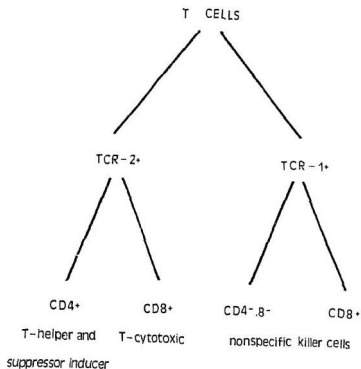
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APPENDICES

Appendix A

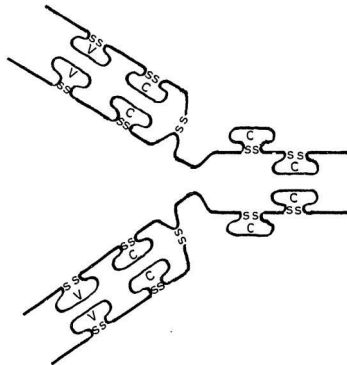


Appendix B



T Cell Differentiation According to Expression of Antigen Receptor TCR-1 or TCR-2 and CD4 or CD8 Surface Molecules.

Appendix C



Basis of Immunoglobulin Structure^{a,b,c}

^aSimilar to a figure in Roitt, 1980.

^bS-S represents a disulfide bond.

^cIncomplete ovals represent domains. These may be constant (C) where considerable homology in amino acid structure is shared between different immunoglobulins or they may be variable (V) when the amino acid sequence of a region is quite different in all immunoglobulins.

Consent for participation in the study

NUTRITIONAL STATUS, IMMUNOCOMPETENCE AND ILLNESS IN THE ELDERLY

Physician Investigator: Dr. R.K. Chandra

I, the undersigned, hereby certify that I have been told about the aims and objectives of the above named study. I understand that a proportion of individuals above the age of 65 years may have nutritional deficiencies. In this study, physical examination and blood tests will be conducted to find out about such deficiencies which will then be corrected by appropriate dietary or medicinal preparations. I understand the possible benefits and discomforts relating to this research study.

I understand that I have the right to ask question about any procedures and to withdraw my consent and stop taking part in the study at any time without assigning any reason and without prejudice to me.

I hereby freely consent to take part in the research study.

Signature

NAME IN BLOCK CAPITALS

Witness

NAME IN BLOCK CAPITALS

Date

Protocol C-83-011

Patient Identification and History: Case Report Form #2

Patient's Initials: _____	Patient No.: _____	Sex (circle): M F	Birth Date: ____/____/____ Day Mo. Yr.
Place of Birth : _____ M.C.P. No.: _____			
Race (circle): (1) Caucasian (2) Other Specify: _____			
Religion: (1) Prot (?) Cath (3) Other Specify: _____			
Marital Status: (1) Married (2) Widowed (3) Single (4) Other (specify): _____			
Highest level of Formal Education Received? (circle)			
(1) Post secondary education. If yes, specify number of years. _____			
(2) Grade XI			
(3) Less than Grade XI. If yes, specify number of years. _____			
(4) Illiterate			
(5) Other (specify): _____			
SOURCE OF INCOME: (circle)			
A. If single:			
(1) Government pension alone			
(2) Government pension + personal income (If yes, which is greater)? _____			
(3) Other (specify): _____			

Investigator's Signature: _____

Date: ____/____/____
Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-011Patient Identification and History: Case Report Form #2 (Cont'd)

Patient's Initials: _____

Patient No.: _____

SOURCE OF INCOME: (Cont'd)

B. If Married or Living with Friend:

- (1) Government pension alone _____
- (2) Government pension + personal income
(If yes, which is greater)? _____
- (3) Government pension of self + spouse (friend) _____
- (4) Government pension + spouse (friend) + personal income
(If yes, which is greater)? _____
- (5) Other (specify): _____

EXERCISE ASSESSMENT:

Does the patient partake in any physical activities?

YES NO

If yes, please specify type of physical activities, duration, frequency.

Type of Activity	Duration	Frequency
	(1) <15 min. (2) 15-30 min (3) 30-60 min. (4) >60 min.	(1) <1/wk. (2) 1-2/wk. (3) 3-5/wk (4) Daily
Walking		
Housework		

Investigator's Signature: _____

Date: ____ / ____ / ____
Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-011Patient Identification and History: Case Report Form #2 (Cont'd)

Patient's Initials: _____	Patient No.: _____																																								
<p><u>EXERCISE ASSESSMENT (Cont'd)</u></p> <p>Are there any reasons, such as physical handicaps, why physical activity or exercise is very restricted? YES NO</p> <p>If yes, specify. _____</p> <p>_____</p> <p>How much time does the patient spend outside? Specify duration in hours per week. _____ Hrs/wk.</p> <p><u>MEDICAL/SURGICAL HISTORY</u> (Check and specify where necessary)</p> <p>Has the patient undergone surgery in the preceeding two years? YES NO</p> <p>If yes, specify: 1 - _____</p> <p style="margin-left: 150px;">2 - _____</p> <p><u>The Patient Presents the Following Medical Conditions:</u></p> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 10px;"> <thead> <tr> <th style="width: 30%;"></th> <th style="width: 15%;">Date of Diagnosis</th> <th style="width: 20%;">Compliance with Treatment (1)Good (2)Fair (3)Poor</th> <th style="width: 30%;">Comments</th> <th style="width: 5%;"></th> </tr> </thead> <tbody> <tr> <td>Cardiac</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> <tr> <td>Respiratory</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> <tr> <td>Metabolic</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> <tr> <td>Hepatic</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> <tr> <td>Gastrointestinal</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> <tr> <td>Orthopedics</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> <tr> <td>Other(s)</td> <td></td> <td></td> <td></td> <td>YES NO</td> </tr> </tbody> </table>			Date of Diagnosis	Compliance with Treatment (1)Good (2)Fair (3)Poor	Comments		Cardiac				YES NO	Respiratory				YES NO	Metabolic				YES NO	Hepatic				YES NO	Gastrointestinal				YES NO	Orthopedics				YES NO	Other(s)				YES NO
	Date of Diagnosis	Compliance with Treatment (1)Good (2)Fair (3)Poor	Comments																																						
Cardiac				YES NO																																					
Respiratory				YES NO																																					
Metabolic				YES NO																																					
Hepatic				YES NO																																					
Gastrointestinal				YES NO																																					
Orthopedics				YES NO																																					
Other(s)				YES NO																																					

Investigator's Signature _____

Nutritional Rehabilitation in the ElderlyProtocol C-83-011Patient Identification and History: Case Report Form #2 (Cont'd)

Patient's Initials: _____	Patient No.: _____
<p><u>MEDICAL/SURGICAL HISTORY</u> (Cont'd)</p> <p>Has there been any change in taste sensation? YES NO</p> <p>Does the patient wear dentures? YES NO</p> <p>If yes, do the dentures cause discomfort or difficulty while eating? YES NO</p> <p>Has there been a dramatic change in weight lately? YES NO</p> <p>If yes, explain. _____</p> <p>Does the patient smoke? YES NO</p> <p>If yes, specify:</p> <p>(1) cigarettes (2) cigars (3) pipe (4) other specify _____</p> <p>Amount smoked per day _____</p> <p>Does the patient consume alcohol? YES NO</p> <p>If yes, specify the amount per week _____</p> <p>Approximately how many bouts of general illness has the patient experienced in the past 6 months (including colds, influenza, etc)?</p> <p>_____</p> <p>_____</p> <p>Has the study candidate received any medication during the past 2 months (including laxatives and vitamin supplements)? YES NO</p> <p>If yes, specify on following sheet.</p>	

Investigator's Signature: _____

 Date: ____/____/____
 Day Mo. Yr.

Patient Identification and History: Case Report Form #2 (Cont'd)

Investigator's Signature: _____
$$I_{\text{eff}} = \frac{1}{2} \frac{1}{M_{\text{eff}}} \frac{1}{Y_{\text{eff}}}$$

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Nutritional Rehabilitation in the Elderly

Protocol C-83-011

Patient Identification and History: Case Report Form #2 (Cont'd)

Patient's Initials: _____	Patient No.: _____
<u>DOMESTIC ENVIRONMENT (Cont'd)</u>	
If Free Living: What type and size is the house? _____	
Are routine cooking appliances available for use?	YES NO
<u>Meal Preparation (circle):</u>	
Meals are prepared by patient.	YES NO
If no, specify by whom meals are prepared. _____	
Are there any foods which the patient strongly dislikes?	YES NO
If yes, specify. _____	

Does the patient have any food allergies?	YES NO
If yes, specify. _____	

Apart from the foods mentioned, are there any other foods that the patient refuses to eat for any reason?	YES NO
If yes, specify. _____	

Appetite (circle): (1) Poor (2) Fair (3) Good	

Investigator's Signature: _____

Date: ____/____/____
Day Mo. Yr.

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Nutritional Rehabilitation in the Elderly

APPENDIX F

Protocol C-83-011

24 Hour Dietary Recall: Case Report Form #3

Patient's Initials: _____	Patient No.: _____	Date: ____/____/____
---------------------------	--------------------	----------------------

(1) Less than normal	(2) Normal	(3) More than normal
----------------------	------------	----------------------

1. Did you eat well yesterday? (circle): (1) (2) (3)
2. Did you eat well today? (circle): (1) (2) (3)

SECTION I: BREAKFAST

3. What was the first thing you had to eat/drink
when you awoke? _____
How much did you eat/drink? _____
4. Do you usually eat breakfast in the morning? YES NO
- Did you eat breakfast yesterday/today? YES NO
- If yes, what time did you begin breakfast?

- Is it the usual time? YES NO
5. What did you eat for breakfast?
A. Fruit or juice YES NO
If yes, specify type and amount. _____
B. Egg(s) YES NO
If yes, specify number and method of
preparation. _____
C. Meat YES NO
If yes, specify type and amount. _____

Investigator's Signature: _____

Date: ____/____/____
Day Mo. Yr.

Protocol C-83-01124 Hour Dietary Recall: Case Report Form #3 (Cont'd)

Patient's Initials: _____	Patient No.: _____
<p>D. Milk, cheese, yogurt or custard YES NO If yes, specify which one and amount. _____</p> <p>E. Cereal or bread YES NO If yes, specify type of bread? _____ Amount _____ What did you have on your bread? _____ Amount _____ Type of cereal? _____ Amount _____ What did you have on your cereal? _____ Amount _____</p> <p>F. Tea, coffee or cocoa YES NO If yes, specify which one(s) and amount. _____ What did you have in your drink? _____</p> <p>G. Did you eat anything else for breakfast? YES NO If yes, specify what and amount. _____</p> <p>6. Did you eat/drink anything after breakfast, before your next meal? If yes, specify what and amount. YES NO _____</p>	
<p><u>SECTION II: MIDDAY MEAL</u></p> <p>7. Do you usually eat a meal in the middle of the day? YES NO Did you eat a meal midday yesterday/today? YES NO If yes, what time did you begin to eat this meal? _____ Is this the usual time? YES NO</p>	

Investigator's Signature: _____

 Date: ____/____/____
 Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-01124 Hour Dietary Recall: Case Report Form #3 (Cont'd)

Patient's Initials: _____	Patient No.: _____
<p>What did you eat at midday?</p> <p>A. Soup YES NO If yes, specify type and amount. _____</p> <p>B. Egg(s) YES NO If yes, specify number and method of preparation. _____</p> <p>C. Vegetables YES NO If yes, specify the type(s) and amount(s). _____</p> <p>D. Meat or fish YES NO If yes, specify which one and amount. _____ Describe the product (i.e., fried cod tongue or baked pork chops) _____</p> <p>E. Casserole YES NO If yes, specify which type and amount. _____</p> <p>F. Fruit or juice YES NO If yes, specify type and amount. _____</p> <p>G. Milk, cheese, yogurt or custard YES NO If yes, specify which one and amount. _____ Describe the product. _____</p>	

Investigator's Signature: _____

 Date:
 (Day Mo. Yr.)

Nutritional Rehabilitation in the ElderlyProtocol C-83-01124 Hour Dietary Recall: Case Report Form #3 (Cont'd)

Patient's Initials: _____	Patient No.: _____
<p>H. Bread, roll, biscuit, cake or cookie YES NO If yes, specify which one and amount _____ Describe the product _____ What did you have on this food? _____</p> <p>I. Tea, coffee or cocoa YES NO If yes, specify which one(s) and amount _____</p> <p>J. Did you eat anything else at this midday meal? YES NO If yes, specify what and amount _____</p> <p>8. Did you eat/drink anything between midday and your next meal? YES NO If yes, specify what and amount _____</p> <p>SECTION III: EVENING MEAL</p> <p>9. Do you usually eat a meal in the late afternoon, at suppertime? YES NO Did your eat supper yesterday/today? YES NO If yes, what time did you begin to eat this meal? _____ Is this the usual time? YES NO</p> <p>A. Soup YES NO If yes, specify type and amount _____</p> <p>B. Egg(s) YES NO If yes, specify number and method of preparation _____</p>	

Investigator's Signature: _____

 Date: ____/____/____
 Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-01124 Hour Dietary Recall: Case Report Form #3 (Cont'd)

Patient's Initials: _____	Patient No.: _____
C. Vegetables If yes, specify the type(s) and amount(s) _____	YES NO
D. Meat or fish If yes, specify which one and amount _____ Describe the product (i.e., fried cod tongues or baked pork chops) _____	YES NO
E. Casserole If yes, specify which type and amount _____	YES NO
F. Fruit or juice If yes, specify type and amount _____	YES NO
G. Milk, cheese, yogurt or custard If yes, specify type and amount _____ Describe the product _____	YES NO
H. Bread, roll, biscuit, cake or cookie If yes, specify which one and amount _____ Describe the product _____ What did you have on this food? _____	YES NO
I. Tea, coffee or cocoa If yes, specify which one(s) and amount _____	YES NO
J. Did you eat anything else at this supper? If yes, specify what and amount _____	YES NO
10. Did you eat/drink anything after supper and before going to bed? If yes, specify what and amount _____	YES NO

Investigator's Signature: _____

Date: / /
Day Month Year

NUTRITIONAL REHABILITATION IN THE ELDERLY

Protocol C-83-011 - Anthropometric Measurements: Case Report Form # 58

		Patient No.:										
Sex	Age	Normal Values	Day 0				ENSURE				FOLLOW-UP	
			2 months	4 months	6 months	8 months	12 months	12 months				
Wt	60-69	156-174 cm										
	70-79	144-172 cm										
	80-89	144-168 cm										
Ht	60-69	46.9-104.5kg										
	70-79	42.1- 95.7kg										
	80-89	39.0- 87.0kg										
Percent Ideal Weight												
Percent Total Body Fat												
Upper Arm Circumference	60-69	230-410 mm										
	70-79	221-369 mm										
	80-89	205-333 mm										
Triceps Skinfold Thickness	60-69	3.1-33.1 mm										
	60-69	3.5-32.7 mm										

Investigator's Signature: _____

Date: _____

APPENDIX G

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Nutritional Rehabilitation in the Elderly

Protocol C-83-011

Biochemistry Record: Case Report #59

APPENDIX II

Patient's Initials: _____		Patient No.: _____			
	NORMAL RANGE	DAY 0	3 MONTHS	9 MONTHS	15 MONTHS
Date (Day/Mo./Yr.)	___/___/___	___/___/___	___/___/___	___/___/___	___/___/___
Months in Study					
Albumin	35 - 70 g/L				
Prealbumin	0.25 - 0.40 g/L				
Serum Ferritin	M 23 - 320 µg/L F 10 - 115 µg/L				
Serum Vit B ₁₂	135 - 710 pmol/L				
Serum Folate	4.5 nmol/L				
C Reactive Protein					
Zinc	11.5 - 18.5 µmol/L				
Complement C ₃	0.55 - 1.20 g/L				
T lymphocytes	0.70 - 0.80				
Other: (specify) _____					

Investigator's Signature: _____

Date: ___/___/___
Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-011Hematology Record: Case Report #60

Patient's Initials: _____		Patient No.: _____			
	NORMAL RANGE	DAY 0	3 MONTHS	9 MONTHS	15 MONTHS
Date (Day/Mo./Yr.)		__/__/__	__/__/__	__/__/__	__/__/__
Months in Study					
Hemoglobin	M 140 - 180 g/L F 120 - 160 g/L				
Hematocrit	M 0.42 - 0.52 F 0.37 - 0.47				
White blood cell count	4.8-10.8 x10 ⁹ /L				
Differential Segmented (neutrophils, polys.)	0.40 - 0.75				
Bands (Stab, juvenile)	< 0.05				
Lymphocytes	0.20 - 0.45				
Monocytes	0.02 - 0.10				
Eosinophils	0.01 - 0.06				
Basophils	< 0.01				
Red Cell Morphology 1 = Normal 2 = Abnormal; Specify	[1] [2] Specify	[1] [2] Specify	[1] [2] Specify	[1] [2] Specify	[1] [2] Spec
Other:					
Other:					
Other:					
Other:					

Investigator's Signature: _____

Date: __/__/__
Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-011

Physical Examination: Case Report form #14

APPENDIX 1

Patient's Initials: _____		Patient No.: _____		Exam. date: ____/____/____	
	SEVERITY			DURATION Day, Week, Month?	COMMENTS
	Mild	Mod.	Severe		
HAIR					
Dull and dry	() 1	() 2	() 3		
Thin and sparse	() 1	() 2	() 3		
Dyspigmented	() 1	() 2	() 3		
Easily pluckable	() 1	() 2	() 3		
Other: _____	() 1	() 2	() 3		
_____	() 1	() 2	() 3		
FACE					
Paleness	() 1	() 2	() 3		
Nasolabial seborrhea	() 1	() 2	() 3		
Other: _____	() 1	() 2	() 3		
_____	() 1	() 2	() 3		
EYES					
Corneal clouding	() 1	() 2	() 3		
Bitot's spots	() 1	() 2	() 3		
Delayed dark adaptation	() 1	() 2	() 3		
Pale conjunctiva	() 1	() 2	() 3		
Xerophthalmia	() 1	() 2	() 3		
Other: _____	() 1	() 2	() 3		
_____	() 1	() 2	() 3		

* If there is no answer, the condition is not present.

Investigator's Signature: _____

Date: ____/____/____
Day, Mo., Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-011Physical Examination: Case Report Form #14 (Cont'd)

Patient's Initials: _____		Patient No.: _____			
	SEVERITY			DURATION Day, Week, Month?	COMMENTS
	Mild	Mod.	Severe		
MOUTH					
Lesions at corners of mouth	() ₁	() ₂	() ₃		
Missing teeth	() ₁	() ₂	() ₃		
Swollen gums	() ₁	() ₂	() ₃		
Bleeding of gums	() ₁	() ₂	() ₃		
Other: _____	() ₁	() ₂	() ₃		
_____	() ₁	() ₂	() ₃		
NECK					
Thyroid enlargement	() ₁	() ₂	() ₃		
Other: _____	() ₁	() ₂	() ₃		
_____	() ₁	() ₂	() ₃		
SKIN					
Thin and shiny	() ₁	() ₂	() ₃		
Dry and scaling	() ₁	() ₂	() ₃		
Petechiae	() ₁	() ₂	() ₃		
Follicular hyperkeratosis	() ₁	() ₂	() ₃		
Non-healing wounds	() ₁	() ₂	() ₃		
Excessive bruising	() ₁	() ₂	() ₃		
Other: _____	() ₁	() ₂	() ₃		
_____	() ₁	() ₂	() ₃		

* If there is no answer, the condition is not present.

Investigator's Signature: _____

Date: ____/____/____
Day Mo. Yr.

Nutritional Rehabilitation in the Elderly

Protocol C-83-011

Physical Examination: Case Report Form #14 (Cont'd)

Patient's Initials: _____		Patient No.: _____			
	SEVERITY			DURATION	COMMENTS
	Mild	Mod.	Severe	Day, Week, Month?	
MUSCULOSKELETAL					
Muscle wasting	()	1 ()	2 ()	3 ()	
Growth retardation	()	1 ()	2 ()	3 ()	
Epiphyseal swelling	()	1 ()	2 ()	3 ()	
Bone pain & tenderness	()	1 ()	2 ()	3 ()	
Persistent fractures	()	1 ()	2 ()	3 ()	
Gen. muscle weakness	()	1 ()	2 ()	3 ()	
Other: _____	()	1 ()	2 ()	3 ()	
_____	()	1 ()	2 ()	3 ()	
NEUROLOGICAL					
Paralysis	()	1 ()	2 ()	3 ()	
Apathy	()	1 ()	2 ()	3 ()	
Mental confusion	()	1 ()	2 ()	3 ()	
Ataxia	()	1 ()	2 ()	3 ()	
Other: _____	()	1 ()	2 ()	3 ()	
_____	()	1 ()	2 ()	3 ()	
LYMPHATIC					
Enlarged tonsils	()	1 ()	2 ()	3 ()	
Enlarged lymph nodes	()	1 ()	2 ()	3 ()	
Other: _____	()	1 ()	2 ()	3 ()	
_____	()	1 ()	2 ()	3 ()	
GENERAL APPEARANCE					
Edema	()	1 ()	2 ()	3 ()	
Obesity	()	1 ()	2 ()	3 ()	
Cachexia	()	1 ()	2 ()	3 ()	
Other: _____	()	1 ()	2 ()	3 ()	
_____	()	1 ()	2 ()	3 ()	

* If there is no answer, the condition is not present.

Investigator's Signature: _____

Date: _____
Day, Mo., Yr.

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Nutritional Rehabilitation in the Elderly

Protocol C-83-011

Morbidity: Case Report Form #15 APPENDIX J

Patient's Initials: _____ Patient No.: _____ Date: ____/____/____
Day Mo. Yr.

1- Body temperature _____ °C

2- Have you experienced any illness in the past two weeks YES NO

If yes, specify _____

	SEVERITY			DURATION	
	Mild	Mod.	Severe	Date Appeared (Day/Mo./Yr.)	Date Ended (Day/Mo./Yr.)
SKIN					
Rash	() ₁	() ₂	() ₃	____/____/____	____/____/____
Fever	() ₁	() ₂	() ₃	____/____/____	____/____/____
Infection	() ₁	() ₂	() ₃	____/____/____	____/____/____
EYES					
Watering	() ₁	() ₂	() ₃	____/____/____	____/____/____
Infection	() ₁	() ₂	() ₃	____/____/____	____/____/____
RESPIRATORY TRACT					
Nasal discharge	() ₁	() ₂	() ₃	____/____/____	____/____/____
Sinus congestion	() ₁	() ₂	() ₃	____/____/____	____/____/____
Cough	() ₁	() ₂	() ₃	____/____/____	____/____/____
Sore throat	() ₁	() ₂	() ₃	____/____/____	____/____/____
Difficult breathing	() ₁	() ₂	() ₃	____/____/____	____/____/____
Infection	() ₁	() ₂	() ₃	____/____/____	____/____/____
GASTROINTESTINAL TRACT					
Nausea	() ₁	() ₂	() ₃	____/____/____	____/____/____
Vomiting	() ₁	() ₂	() ₃	____/____/____	____/____/____
Diarrhea	() ₁	() ₂	() ₃	____/____/____	____/____/____
Infection	() ₁	() ₂	() ₃	____/____/____	____/____/____
URINARY TRACT					
Painful urination	() ₁	() ₂	() ₃	____/____/____	____/____/____
More frequent urination	() ₁	() ₂	() ₃	____/____/____	____/____/____
Infection	() ₁	() ₂	() ₃	____/____/____	____/____/____

* If there is no answer, condition is not present.

Investigator's Signature: _____

Date: ____/____/____
Day Mo. Yr.

Nutritional Rehabilitation in the ElderlyProtocol C-83-011

APPENDIX E

Delayed Hypersensitivity Record: Case Report #61

Patient's Initials: _____		Patient No.: _____		
	DAY 0	6 MONTHS	12 MONTHS	
Date (Day/Mb./Yr.)	___/___/___	___/___/___	___/___/___	___/___/___
Months in Study				
Date Planted (Day/Mb./Yr.)	___/___/___	___/___/___	___/___/___	___/___/___
Date Read (Day/Mb./Yr.)	___/___/___	___/___/___	___/___/___	___/___/___
Trichophyton Induration Diameter (mm)				
Candidin Solution (1000 PMU/mL) Induration Diameter (mm)				
Purified Protein Derivative (5 Tuberculin Units) Induration Diameter (mm)				
Phytohemagglutinin Induration Diameter (mm)				
Tetanus Toxoid (0.1 Lf units) Induration Diameter (mm)				
Control Induration Diameter (mm)				

Investigator's Signature: _____

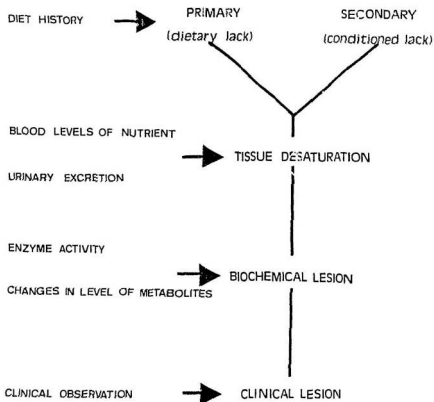
Date: ___/___/___
Day Month Year

APPENDIX L

COMPOSITION OF ENSURE PLUS

volume	235 ml.
energy value	355 kcal.
protein	12.9 g.
fat	12.5 g.
carbohydrate	46.9 g.
vitamin A	620 IU (186.2 RE)
vitamin D	47.0 IU (1.2 mcg.)
vitamin E	11.2 IU (7.5 mg.)
vitamin K ₁	49.6 mcg.
vitamin C	37.68 mg.
folacin	0.05 mg.
thiamin	0.62 mg.
riboflavin	0.64 mg.
niacin	7.44 mg.
pyridoxine	0.74 mg.
vitamin B ₁₂	2.07 mcg.
biotin	0.07 mg.
pantothenic acid	1.98 mg.
sodium	0.25 g. (10.87 mmol.)
potassium	0.45 g. (11.54 mmol.)
chlorine	0.38 g. (10.70 mmol.)
calcium	0.15 g.
phosphorus	0.15 g.
magnesium	0.07 g.
iodine	24.91 mcg.
manganese	0.50 mg.
copper	0.38 mg.
zinc	5.58 mg.
iron	3.35 mg.

METHOD OF STUDY

Appendix M**Development of a Nutritional Deficiency^a**

^aSimilar to a figure in Krause and Mahan, 1984.



